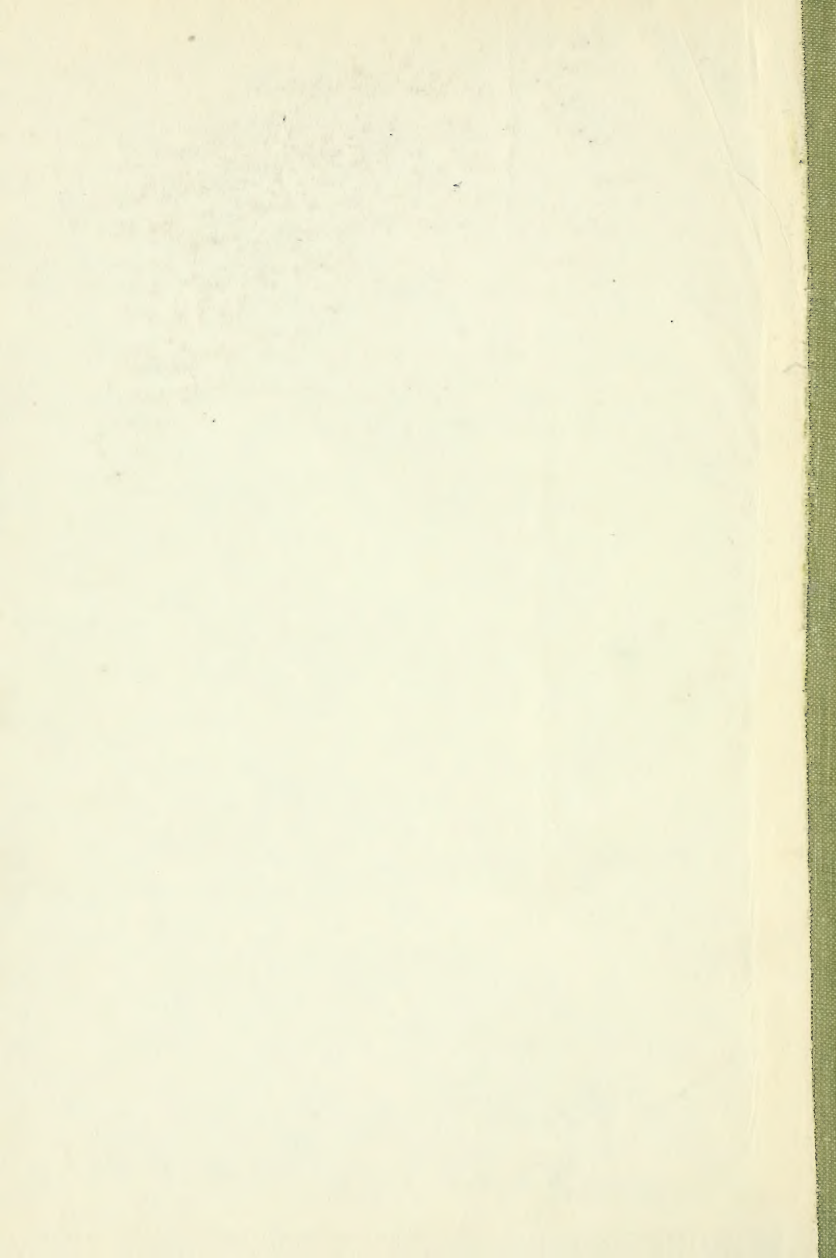
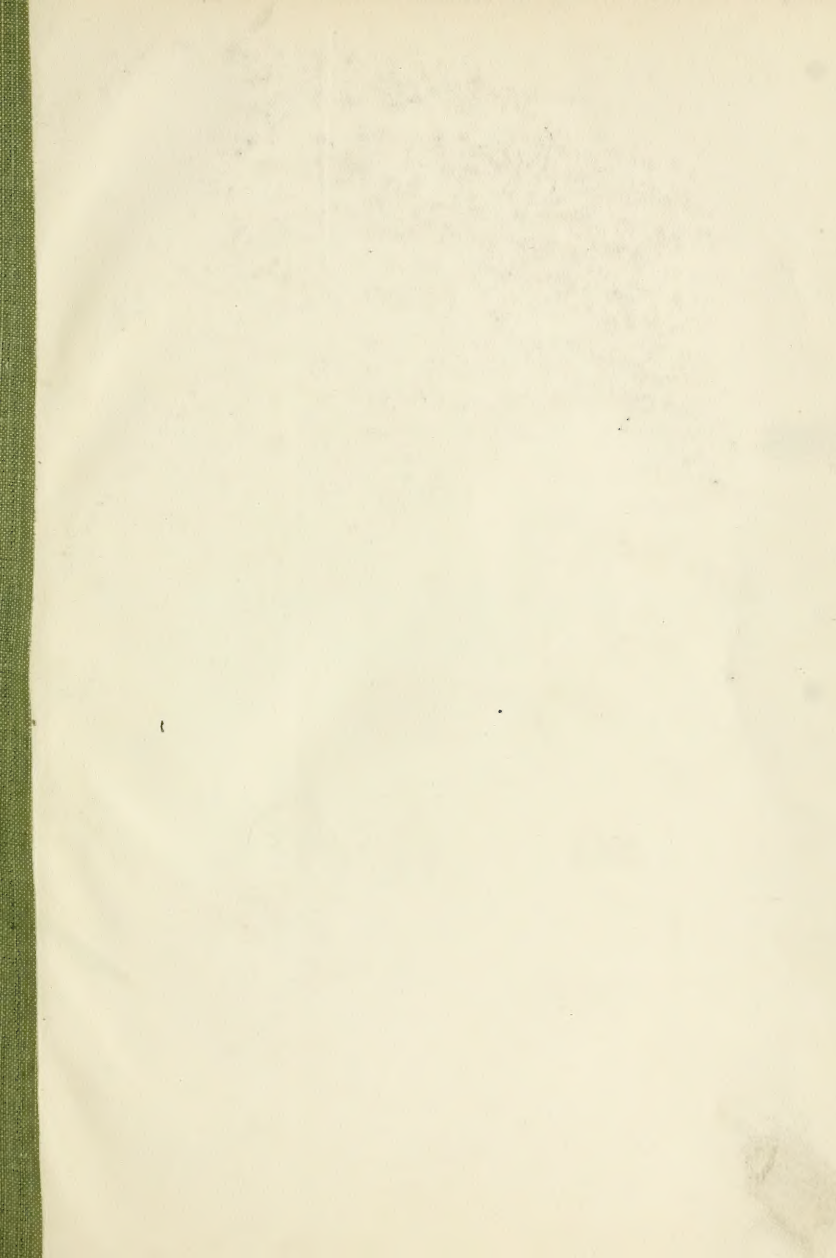



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# RECORDS OF THE OCCURRENCE OF INTESTINAL PROTOZOA IN BRITISH AND INDIAN TROOPS IN MESOPOTAMIA.

BY

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[Received for publication, February 22, 1919.]

## INTRODUCTION.

SINCE 1915 when dysentery convalescents first came streaming back from Gallipoli and other eastern war zones, a very large number of laboratory workers have recorded their protozoological findings in cases of intestinal disorders among the troops. One is therefore somewhat reluctant to burden the literature with further statistics of this kind. A great proportion, however, of the published work deals only with convalescent cases at home, in India, or other places remote from the war zones, and is moreover largely restricted to British cases. So far, I believe, there has been no published record of protozoological work of this nature carried out in Mesopotamia, and I know only of one paper in which any comparison is made between the findings in British and Indian cases: Woodcock (1917) (1) in Egypt found a marked disparity between British and Indian stools as regards the occurrence of protozoan infections, and the object of the present paper is to record the comparative findings in different series of dysentery and diarrhoea cases among British and Indian troops in Mesopotamia, 1916-18.

I am well aware that the actual value of percentages of the kind given below is small. The personal factor in such examinations is an important one, and it is also now well known that one or two examinations of any case are quite inadequate to detect all protozoan infections. This applies especially to encysted forms in convalescent or non-acute cases, my experience with acute dysenteries being that the free forms of *Entamoeba histolytica* are nearly always found on the first examination,

if the case is actually one of amœbic dysentery : hence the figures given for this class of case are certainly considerably lower than they would have been if a more complete investigation had been made. It is therefore only to the comparative percentages in the different series, British and Indian, that I attach any importance, the examinations in all cases having been made in the same country by one observer using similar methods throughout.

# I. FINDINGS IN BRITISH CASES.

The majority of British cases dealt with in this section were examined by me whilst I was attached to a British General Hospital in Basra from September 1916 to December 1917. The average number of examinations made in each case was 1·5, a very large number of patients having been examined once only owing to their having been transferred to India or elsewhere before further investigations could be made.

The total number of these cases was 1,378 : they were a mixed lot, consisting of acute dysenteries (*i.e.*, with blood and mucus in the stools), colitis and diarrhœa cases, as well as of a number of convalescents passing more or less normal stools.

The findings in this mixed series are given in the following table :—

## BRITISH CASES.

### SERIES I : DYSENTERY, COLITIS, DIARRHŒA, ETC.

*Total Number of Cases*                      ..                      ..                      1,378.

Protozoa.				Number of cases infected.
<i>Entamœba histolytica</i> ..	..	..	..	201 = 14·5%
<i>Entamœba coli</i> ..	..	..	..	264 = 19·1%
<i>Giardia (Lambia) intestinalis</i> ..	..	..	..	133 = 9·7%
<i>Trichomonas intestinalis</i> ..	..	..	..	111 = 8 %
<i>Chilomastix (Tetramitus) mesnili</i> ..	..	..	..	68 = 5 %
<i>Coccidium (Isospora)</i> ..	..	..	..	8 = ·6%

*Entamœba nana* is not included in the table. During the earlier part of the investigation this species was confused with *Amœba limax* and its cysts were unidentified, the form being not definitely recognised until Wenyon's paper (2) containing the description of the new species was received during the summer of 1917. In a series of 300 cases examined in the autumn of that year, *Entamœba nana* was found in 19, *i.e.*, 6·3 per cent. In addition to the forms mentioned above, unidentified flagellates (*Prowazekia* ?) were found on two occasions.



In order to obtain a more accurate idea as to the nature of the dysentery cases, the acute dysenteries were separated from the other forms of intestinal disorders in Series I. For this purpose I have regarded as 'acute dysenteries' all cases in which the stools showed blood and mucus macroscopically, or were found to contain both pus cells and red blood cells when examined under the microscope. The findings of *Entamæba histolytica* in this series are shown below:—

## BRITISH CASES.

## SERIES II: ACUTE DYSENTERIES.

<i>Total Number of Cases</i>	..	..	442.
Protozoa.			Number of cases infected.
<i>Entamæba histolytica</i>	..	..	110 = 24·8%

These figures, therefore, show that nearly 25 per cent of the acute dysentery cases among British troops in Mesopotamia were of amoebic origin.

The various protozoa included in these lists consisted of course both of free and encysted forms. In order to compare the occurrence of these different forms of the pathogenic and non-pathogenic species, the following analysis was made of all the amoebic findings in the two series:

## ANALYSIS OF THE AMOEBIC FINDINGS IN SERIES I AND II.

	Acute cases, with blood and mucus.	Cases without blood and mucus.	Total in all cases.
<i>E. histolytica</i> all forms ..	110	91	201
.. .. free forms, with or without cysts ..	99	44	143
.. .. free forms, without cysts ..	82	17	99
.. .. cysts, with or without free forms ..	28	74	102
.. .. cysts, without free forms ..	11	47	58
.. .. cysts, with free forms ..	17	27	44
<i>E. coli</i> all forms ..	66	198	264
.. .. free forms, with or without cysts ..	25	37	62
.. .. free forms, without cysts ..	10	15	25
.. .. cysts, with or without free forms ..	56	183	239
.. .. cysts, without free forms ..	41	161	202
.. .. cysts, with free forms ..	15	22	37

The analytical table is self-explanatory. It must, however, be remembered that the figures refer to cases and not to stools. Thus where free forms are shown as occurring with cysts, it does not necessarily mean that these were found in the same stool; the two forms may have been diagnosed during different examinations of the same case. The use of the table will be made clearer if we consider the *Entamoeba histolytica* infections in the acute cases (first column). The table shows that out of 110 infections in acute dysenteries 99 were diagnosed from the free-living active forms of the *Entamoeba*; in the remaining 11 cases free forms were not found in the blood and mucus, but *Entamoeba histolytica* was diagnosed from cysts, either found in the more solid parts of the same stool, or during later examinations of the case.

For comparison with the findings in cases of dysentery and other intestinal disorders, it was considered of interest to obtain corresponding figures for healthy British troops in Mesopotamia, or rather from men not suffering from intestinal troubles, since such an investigation could best be carried out in a hospital. A series of 200 men were examined in Baghdad; they consisted partly of surgical cases and partly of 'non-intestinal' medical cases. The protozoological findings were as follows:—

#### BRITISH CASES.

##### SERIES III: NON-INTESTINAL CASES.

Total Number of Cases				200.
Protozoa.				Number of cases infected.
1				
<i>Entamoeba histolytica</i> ..	..	..	..	13 = 6.5%
<i>Entamoeba coli</i> ..	..	..	..	42 = 21 %
<i>Entamoeba nana</i> ..	..	..	..	12 = 6 %
<i>Giardia (Lamblia) intestinalis</i> ..	..	..	..	17 = 8.5%
<i>Trichomonas intestinalis</i> ..	..	..	..	5 = 2.5%
<i>Chilomastix (Tetramitus) mesnili</i> ..	..	..	..	8 = 4 %

Each case was examined once only. The percentages are therefore certainly lower than they would have been if more exhaustive investigation of the cases had been possible. When compared with the figures in Series I, we find the percentage of *Entamoeba histolytica* considerably less than half that found in the cases of dysentery and diarrhoea; on the other hand the figures for *Entamoeba coli* and *Lamblia* do not appreciably differ in the two series. Since the material consisted chiefly of formed stools, the percentage for *Trichomonas* is naturally low. *Chilomastix mesnili* in this series consisted largely of encysted forms.

## II. FINDINGS IN INDIAN CASES.

Of the cases included in this section a few were examined in the Basra area in 1916; the majority, however, were investigated during a tour through the country during the winter 1917-18. The average number of examinations of each case was 1·2 only, as a very large number were examined only once. The total cases numbered 906. For the purpose of comparison I have arranged these in similar series to those in the British section.

## INDIAN CASES.

## SERIES I: DYSENTERY, COLITIS, DIARRHŒA, ETC.

Total Number of Cases .. .. 906.

Protozoa.	Number of cases infected.
<i>Entamœba histolytica</i> .. .. .	188 = 20·7%
<i>Entamœba coli</i> .. .. .	276 = 30·4%
<i>Entamœba nana</i> .. .. .	90 = 9·9%
<i>Giardia (Lambdia) intestinalis</i> .. .. .	74 = 8·1%
<i>Trichomonas intestinalis</i> .. .. .	96 = 10·6%
<i>Chilomastix (Tetramitus) mesnili</i> .. .. .	30 = 3·3%
<i>Coccidium (Isospora)</i> .. .. .	1 = 0·1%

As in the previous section, the acute dysenteries were separated from the mixed cases of intestinal disorders in Series I, and the *Entamœba histolytica* infections in these acute cases are tabulated below:—

## INDIAN CASES.

## SERIES II: ACUTE DYSENTERIES.

Total Number of Cases .. .. 135.

Protozoa.	Number of cases infected
<i>Entamœba histolytica</i> .. .. .	65 = 48·1%

Among Indians, we find therefore a much higher percentage of *Entamœba histolytica* infections in the acute dysenteries than in the corresponding series of British cases, nearly one-half of the acute dysenteries among Indians being of amoebic origin.

As in the British section a control series of non-intestinal cases were also investigated; these consisted of 200 surgical and non-intestinal medical patients from one of the Indian hospitals in Baghdad. Each case was examined once only.

## INDIAN CASES.

## SERIES III: NON-INTESTINAL CASES.

Total Number of Cases				200.
Protozoa.				Number of cases infected.
<i>Entamæba histolytica</i> ..	..	..	..	21 = 10·5%
<i>Entamæba coli</i> ..	..	..	..	81 = 40·5%
<i>Entamæba nana</i> ..	..	..	..	18 = 9 %
<i>Giardia (Lamblia) intestinalis</i> ..	..	..	..	16 = 8 %
<i>Trichomonas intestinalis</i> ..	..	..	..	8 = 4 %
<i>Chilomastix (Tetramitus) mesnili</i> ..	..	..	..	6 = 3 %

As was to be expected, we here again see a considerable falling off in the figures for *Entamæba histolytica*, the percentage being about half that in the series of mixed cases. On the other hand the percentage of *Entamæba coli* is higher, that of *Entamæba nana* and *Lamblia* much the same as in Series I.

## III. COMPARISON BETWEEN FINDINGS IN BRITISH AND INDIAN CASES.

As mentioned in the introduction, Woodcock found a marked disparity between the findings in British and Indian cases in Egypt. It is of interest therefore to compare the results obtained by me in the two sections. The difference between British and Indians is most marked as regards the *Entamæba histolytica* findings as shown by the following table :—

PERCENTAGES OF *Entamæba histolytica*.

			Non-intestinal cases.	Cases of Dysentery, Colitis and Diarrhœa.	Acute Dysentery cases.
			%	%	%
British	..	..	6·5	14·5	24·8
Indian	..	..	10·5	20·7	48·1

In each of the series the percentage of *Entamæba histolytica* is markedly higher among Indians than among British; the difference is most noticeable in the acute dysentery series, where the percentage for Indians is twice that for British.

The figures for *Entamæba coli* are also considerably higher in the Indian series than in the British.

The percentages of *Giardia (Lamblia) intestinalis* are remarkably constant in the two classes of patients. *Trichomonas* however seems somewhat commoner among Indians than among British, but the difference is not nearly as great as found by Woodcock in Egypt. Curiously enough, *Chilomastix (Tetramitus)* throughout the investigation was found more abundantly among the British patients.

#### IV. NOTES ON THE SPECIES OF PROTOZOA.

So much has been written recently on the differences between *Entamoeba histolytica* and *Entamoeba coli* that I need not refer to the morphology of the two species. It is necessary, however, to mention that, during the investigations dealt with in this paper, in the absence of characteristic cysts, no amoeba was diagnosed as *Entamoeba histolytica* unless seen in the motile condition and containing red blood cells.

Matthews (1918) (3) has recently given an excellent summary of the differential characters of the cysts in *Entamoeba histolytica* and *Entamoeba coli* and I have nothing to add to this account. It is now well established that there is great variation in the size of the cysts of these two species of *Entamoeba*. In Mesopotamia I found the cysts of *Entamoeba histolytica* to vary in diameter from  $6\mu$ – $18\mu$ ,—a somewhat smaller range of variation than found by some recent observers. *Entamoeba coli* was found to vary from  $9\mu$ – $31\mu$ ; on one occasion a small strain of this species was met with in which no cyst measured over  $14\mu$ , the smallest being  $9\mu$  in diameter; the majority of these cysts were in the 8-nucleated stage and quite characteristic of the species.

The cysts of *Giardia (Lamblia) intestinalis* were also found capable of great variation in size; my records of measurements show all lengths from  $9\mu$  to  $16.5\mu$ .

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# REPORT ON BILHARZIASIS IN MESOPOTAMIA.

BY

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[Received for publication, February 22, 1919.]

## INTRODUCTION.

EVIDENCE of the occurrence of bilharziasis among the native population of Mesopotamia was first published by Sturrock in 1899; no further information on this subject has, however, been available since that date, and during recent years it has been generally believed that the disease in that country was restricted to the swampy regions of the Lower Euphrates, and absent from the Basra area and the inhabited districts of the Tigris valley.

This belief seemed to be confirmed by the general absence of the disease among British and Indian troops in Mesopotamia during the first years of our occupation, the parasite having been found only twice up to the autumn of 1917, in both cases in Indian Sepoys stationed in the Nasiriyeh district on the Euphrates.

At the beginning of November 1917, a small outbreak of bilharziasis among the personnel of an Indian General Hospital at Basra drew attention to the importance of the subject from the point of view of military sanitation, and I was directed, first to report upon the origin of this outbreak; and later, by means of a rapid survey, to investigate the prevalence of the disease among the Arab population of the occupied districts. At the same time a malacological survey was made of both

the Tigris and Euphrates areas in the hope of discovering the molluscan intermediary host of the parasite.

The results of my investigations were reported at intervals to the Director of Medical Services, Mesopotamian Expeditionary Force. The present paper gives a summary of these observations; it has been thought advisable to present the facts in more or less chronological order, commencing with an account of the enquiry into the outbreak which first drew attention to the prevalence of the disease.

#### I. AN OUTBREAK OF BILHARZIASIS AMONG THE PERSONNEL OF AN INDIAN GENERAL HOSPITAL AT BASRA.

The attention of the medical staff was first attracted to the disease by a case of hæmaturia in an Indian *bhisti* employed in the hospital. The urine of the patient was brought to me for examination and the terminal-spined ova of *Schistosoma hæmatobium* were found in large numbers.

A second case was discovered a few days later (November 1917), and, acting on the suggestion that these were not likely to be isolated cases, the Officer in charge of the Hospital Laboratory, Captain J. McKerchar, R.A.M.C., inspected others of the personnel with positive results, finally submitting the urines of the whole Indian staff to microscopical examination, with the result that 71 out of 251, *i.e.*, roughly 28 per cent, were found to be infected with *Schistosoma*. In all cases the parasite was *S. hæmatobium*.

No infections were found among the small number of British personnel employed in the hospital.

In order to arrive at any conclusion as to the origin of this widespread infection among the Indian personnel, it was necessary to consider the following important ætiological factors:—

(1) The hospital was situated in the Ashar suburb of Basra, a few hundred yards from the Shatt-el-Arab, with its main frontage on the left bank of the Khandaq Creek, one of the four principal canals which intersect the city area and open into the main river. The *dhobie ghat* of the hospital was on the creek just below an important ferry, a favourite spot with the Arab population for drawing water, washing, etc., hence very liable to pollution.

At the back of the hospital were large palm-groves, extensively flooded from the creek during the early summer, and at the date of the



enquiry still containing numerous large pools overgrown with water weeds.

Opposite the hospital, on the right bank of the creek, were the sites of various Labour Corps camps, one of them recently occupied by the



FIG. I.

Khandaq Creek, Basra.

Egyptian Labour Corps. The Indian personnel had been in the habit of bathing and washing in the Khandaq Creek during the summer months.

(2) The following detail of the personnel infected with *Schistosoma* shows that infection was not restricted to any particular caste or occupation. I expected to find the highest percentage of infection among the *dhobies* and *bhisties*; these were heavily infected, but the highest percentage was found among the men employed in the cook-houses :—

*Cooks—*

Total No.	..	..	..	..	14
.. .. infected	..	..	..	..	9

*Dhobies—*

Total No.	..	..	..	..	12
„ „ infected	..	..	..	..	8

*Bhisties—*

Total No.	..	..	..	..	18
„ „ infected	..	..	..	..	6

*Sweepers—*

Total No.	..	..	..	..	40
„ „ infected	..	..	..	..	13

*Dooli Bearers—*

Total No.	..	..	..	..	89
„ „ infected	..	..	..	..	20

*Dressers and Ward Orderlies—*

Total No.	..	..	..	..	48
„ „ infected	..	..	..	..	8

*Storekeepers—*

Total No.	..	..	..	..	8
„ „ infected	..	..	..	..	2

*Mistries—*

Total No.	..	..	..	..	2
„ „ infected	..	..	..	..	1

*Guides—*

Total No.	..	..	..	..	4
„ „ infected	..	..	..	..	1

*Tailors—*

Total No.	..	..	..	..	2
„ „ infected	..	..	..	..	0

*Bullock drivers—*

Total No.	..	..	..	..	6
„ „ infected	..	..	..	..	1

*Havildars—*

Total No.	..	..	..	..	2
„ „ infected	..	..	..	..	1

*Clerks—*

Total No.	..	..	..	..	6
„ „ infected	..	..	..	..	1

(3) At my request Captain McKerchar obtained particulars of the histories of the various infected individuals prior to their arrival in Mesopotamia. Only 5 out of the 71 had been out of India before : one had been in France, one had spent six months at Muscat, and three had been on service in Egypt during the War.

The infected individuals belonged to a number of different Indian races and had been recruited from widely separated districts; they included several Gurkhas. The dates of their arrival in Mesopotamia varied between January 1916 and August 1917. The very large majority were detailed to the Basra Hospital within a few weeks of their arrival in the country and had never left the Base Area.

(4) Reliable information as to the nature of symptoms and the dates of their first appearance is notoriously difficult to obtain from Indian patients. The Assistant Surgeons at the hospital were, however, able to prepare a series of case sheets, from which a certain number of facts were available:—13 of the infected men (including two of those who had served in Egypt) had no symptoms at all: the remainder stated that blood was occasionally passed with the urine and complained of pains or a 'burning sensation' during micturition.

With regard to the dates of the first appearance of these symptoms, a large majority (including the third man who had been in Egypt) professed to have first noticed them in October or early November 1917, *i.e.*, a few weeks before the enquiry. However unreliable these facts may have been, I think they were sufficient for us to accept the view that the infections were of recent origin. The incubation period of bilharziasis is known to be short; we can therefore assume that the majority of infections in the hospital were acquired some time during the summer of 1917.

The facts recorded in the preceding paragraphs all suggested that the parasitized personnel of the hospital had acquired the disease locally. Before seeking the exact source of the infections, it was, however, thought advisable to ascertain whether these were restricted to this single Indian hospital, or whether bilharziasis was prevalent in other units at Basra.

There seems to be universal agreement that the *Schistosoma* parasite is not commonly found in India, and that the occasional cases of bilharziasis recorded in that country are to be considered as imported from other countries where the disease is endemic.

As a control experiment I examined 100 of the Indian personnel of two other hospital units in the Base Area, including 56 *dhobies* and 35 *blishties*; none of them proved to be infected. These figures, although small, are sufficient to show that the infection was not a widespread one among Indians in Basra, and that the infections at the hospital had most probably been acquired in the immediate neighbourhood.

The fact that all classes of the Indian personnel had been in the habit of bathing and washing in the Khandaq Creek, and that the hospital *dhobie ghats* were also situated there, pointed definitely to this creek as the source of the *Schistosoma* infection.

Wherever bilharziasis occurs, infection is rapidly spread owing to the fact that natives are in the habit of passing urine and fæces into ponds, canals, irrigation creeks, etc., which harbour the intermediate molluscan host of the parasite.

Since the disease was not endemic among the personnel of the hospital, two possible sources of infection suggested themselves



FIG. II.

Map of Mesopotamia, showing localities mentioned in the Report. The shaded areas show the marshy districts.

immediately: (a) the Egyptian Labour Corps recently encamped in the neighbourhood of the Khandaq Creek; (b) the native Arab population of Basra and Ashar.

(a) The Egyptian Labour Corps was situated close to the right bank of the Khandaq Creek at no great distance from the hospital. The camp had, indeed, no frontage on the creek, yet part at least of its sullage waters would find its way into it; also the Egyptians had free access to the creek.

The widespread prevalence of bilharziasis in Egypt may be gathered from the fact that recent investigators have estimated the incidence of the disease to be between 40 and 70 per cent of the rural population of that country. The Labour Corps consisted largely of 'fellahin' and can therefore be assumed to have been heavily infected with the parasite. No statistics on the subject were available; but enquiries at various hospitals at the Base showed that many cases of bilharziasis were reported among the Egyptians, and that the typical ova were in most cases identified in the laboratories.

(b) As mentioned in the introduction, bilharziasis has long been known to occur among the Arab population in Mesopotamia. I could not, however, obtain any account of its distribution in the country, or ascertain whether cases had been recorded from Basra. I therefore considered it desirable to obtain such information—(i) by enquiry at the Civil Hospitals in the Base Area; (ii) by examination of the urines of local Arabs.

Enquiries at the Civil hospitals were disappointing as no records of bilharziasis cases were found; thanks, however, to the assistance of Capt. F. T. Wood, R.A.M.C., Sanitary Officer for Basra and Ashar. I was able to examine samples of urine from 50 male Arabs, inhabitants of the Ashar district.

Of these 50, nine, *i.e.* 18 per cent. were found to be infected with *Schistosoma hæmatobium*. The Arabs examined were a mixed lot—chiefly, coolies, donkey boys, etc.,—and the diagnoses were made by the quick method without centrifuging. A more complete examination of a more essentially 'amphibious' population, *e.g.*, *bellumchis*, the local boatmen, would probably reveal a still higher percentage.

These figures, although small, were sufficient to show that the Arab population of the Basra district was infected with *Schistosoma* to a far greater extent than was generally assumed. This fact was therefore a sufficient explanation of the infections acquired in a unit situated on the Khandaq Creek adjacent to the town. That the Egyptian Labour Corps may have increased the incidence of the infection is of

course possible, and the arrival of other units from countries where bilharziasis is endemic (*e.g.*, Mauritius and the West Indies) may undoubtedly also have contributed to the infection of the district generally.

## II. THE DISTRIBUTION OF BILHARZIASIS IN MESOPOTAMIA.

The facts recorded in the previous section show that bilharziasis had been acquired by Indian troops in the Basra Area, and that, moreover, the disease is quite common among the Arab population of that district. Although no further outbreaks among the troops had been reported from other parts of Mesopotamia, it seemed important to obtain information as to the distribution of *Schistosoma* in the country, especially with a view to ascertaining whether the disease was prevalent among the Arab inhabitants of the Tigris districts above the junction with the Euphrates, and also of the drier regions in the neighbourhood of the latter river above Baghdad. Such information was obtained by me during a tour in the winter of 1917-18; the Lower Euphrates area in the neighbourhood of Nasiriyeh and Suk-esh-Shuyuk had already been visited early in 1917.

In the various districts evidence of the occurrence of Bilharziasis was obtained both by examination of local natives and by enquiries at Civil Hospitals and Dispensaries, where such were available. During a rapid tour of inspection it was not possible, nor thought necessary, to examine large numbers of Arabs, especially as these are not easy subjects for investigation. Only sufficient were therefore examined to ascertain definitely the presence of the parasite in each district. Great care, however, was taken to select suitable series, and in each locality it was ascertained that the subjects examined were really local inhabitants: these were chosen chiefly from locally recruited Labour Corps and from patients attending Civil Dispensaries. As far as possible boys and youths were selected, partly on account of their predilection for bathing in small pools, canals, etc., partly as being less likely to have travelled far from their districts.

Urine only was examined, and these by the ordinary sedimentation method, without the use of a centrifuge; hence the percentages of infection were probably even larger than given in the table below. The ova found were in all cases the terminal-spined ova of *Schistosoma hæmatobium*.

TABLE SHOWING THE DISTRIBUTION OF SCHISTOSOMA  
HÆMATOBIUM AMONG ARABS IN DIFFERENT DISTRICTS OF  
MESOPOTAMIA.

District.				No. of Arabs examined.	No. of positive findings.	Percentages.
						%
Basra	..	..	..	50	9	18
Kurnah	..	..	..	13	11	85
Amara	..	..	..	30	6	20
Baghdad	..	..	..	24	2	8
Samarra	..	..	..	20	2	10
Felujah	..	..	..	17	6	35
Baqubah-Sharoban	..	..	..	20	..	..
				174	36	20

The table shows that *Schistosoma* infections were found in six out of the seven districts where examinations were made. The swampy area round Kurnah, at the point of junction of the Tigris and the Euphrates, proved to be the most heavily infected. Bilharziasis is also shown to be common along the whole course of the Tigris from Amara up to Samarra, and in the desert district of Felujah on the Euphrates, north-west of Baghdad, a long way from the swampy area. Twenty-two of the Arabs examined in Baghdad were inhabitants of the main city on the left bank, and proved not to be infected; the two parasitized individuals were from the right bank and were cases shown to me in the Civil Hospital by Capt. Carey Evans, I.M.S. The Nasiriyeh district of the Lower Euphrates is not included in the above table; it is, however, known to be heavily infected; and records from the Civil Dispensary



in that town showed that 92 cases of hæmaturia were diagnosed as bilharziasis during the period November 1915 to September 1916.

Of the 36 positive cases shown in the table, only nine showed any symptoms of hæmaturia: the majority can therefore be regarded as 'carrier' cases.

### III. THE MOLLUSCAN INTERMEDIATE HOSTS OF SCHISTOSOMA IN MESOPOTAMIA.

Thanks to the researches conducted in recent years by Japanese parasitologists on *Schistosoma japonicum*, and by Dr. R. T. Leiper in 1915 on the Egyptian species, the life-history of this trematode genus is now well known, various fresh water Gastropoda having been proved to act as the intermediate hosts of the parasites.

In Egypt, Dr. Leiper showed that species of *Bullinus*, especially *B. contortus*, are the second hosts of *Schistosoma hæmatobium* in that country, *Planorbis boissyi* acting in a similar capacity for *S. mansoni*. The molluscan hosts in other countries are being discovered gradually: a second genus of physids *Physopsis* is considered to be the intermediate host of *S. hæmatobium* in Natal, whilst *Planorbis olivaceus* is responsible for the *S. mansoni* infections in some parts of South America.

Since bilharziasis was shown to be quite common among the Arab population of Mesopotamia, it became of the greatest importance to examine the molluscan fauna of that country, with the object of ascertaining whether *Bullinus contortus* and *Planorbis boissyi* occur in the occupied districts, or if other Gastropoda are acting as the second hosts of the parasite in their absence.

Fresh water snails are liable to occur in any permanent or semi-permanent collections of water, so long as these harbour sufficient vegetation to support them. In Mesopotamia, therefore, one finds them most abundantly in marshes, ponds and stagnant creeks. Owing to the absence of vegetation and the swift currents they do not occur in any numbers in the large rivers and main canals leading from them, nor in irrigation channels liable to long periods of desiccation. It follows from this that the districts most liable to harbour Molluscs are those which are marshy or perennially irrigated by a network of creeks and ditches. Since in Mesopotamia nearly all water is derived directly or indirectly from the two great rivers, such conditions occur wherever the river banks are low: e.g., along the Shatt-el-Arab from Kurnah to the Persian Gulf, and along the Lower Euphrates, below Nasiriyeh.

In these regions during the flood season the river water overflows the banks in numerous localities, giving rise to marshes and ponds which remain for the greater part of the year and become overgrown with weeds which harbour an abundant molluscan fauna. Similarly, during the flood season the extensive network of small irrigation canals become filled with water from the main creeks, and may form breeding grounds throughout the year for the hosts of *Schistosoma*.

Being stationed in Basra for a considerable period, I was able to obtain a fairly thorough knowledge of the fresh-water fauna of that area; collections were also made at Kurnah and in the Nasiriyeh-Suk region of the Euphrates. Species of *Bullinus* were specially sought for in these localities, and in this respect the results of my investigations were decidedly disappointing: except in a sub-fossil condition, no specimens of the genus were found there.

Careful attention was given to the Molluscan fauna of Basra during my enquiry into the outbreak of bilharziasis at the Indian General Hospital. Owing to the scarcity of weeds, the rapid flow and the tidal nature of their waters, the Shatt-el-Arab and the main creeks within the city are not very suitable localities for Gastropod Molluscs, species of *Neritina* and *Melanopsis* being the only abundant forms. In the higher reaches of the creeks, and in the numerous pools and irrigation channels communicating with them, vegetation is abundant and supports large numbers of fresh-water snails: a *Limnaea* and a *Planorbis* are extremely abundant in these localities, and with them are found *Melania tuberculata* and *Melanopsis*. The Pelecypoda are represented chiefly by a species of *Corbicula*.

The Euphrates swamps in the neighbourhood of Gurnut Ali, north of Basra, are also affected by tides, and their fauna is similar to that of the Shatt-el-Arab, *Neritina* and *Melanopsis* being the only common genera, whilst the bivalves are abundantly represented by species of *Unio* and *Corbicula*.

The fauna of Kurnah and of the Nasiriyeh-Suk districts of the Euphrates is similar to that of the pools and irrigation channels of Basra: molluscan life is abundant, but the species belong almost exclusively to the genera *Limnaea*, *Planorbis* and *Melania*.

No fresh specimens of *Bullinus* were found in any of the above localities.

Large numbers of specimens of all these genera were dissected including many from the Khandaq Creek area: none were found infected

with bilharzial *Cercaria*. For various reasons I was unable to attempt any infection experiments.

Along the Tigris from Amara up river to Samarra, and in the Euphrates region Felujah-Hit, the conditions differ markedly from those prevailing in Lower Mesopotamia. Here the rivers are provided with relatively high banks, and water for the cultivated areas has to be raised by hand or by means of pumps. This irrigation is carried on extremely intermittently, and hence the irrigation channels are periodically dry, thus preventing the growth of plant life and the development of an abundant molluscan fauna. I found permanent collections of water in the form of ponds and marshes extremely rare.

The Gastropod-fauna of these districts is similar to that of Lower Mesopotamia, but less abundant. A second species of *Limnaea* was found at Amara: here also numerous dry but fresh shells of *Bullinus contortus* were found in a recently dried irrigation channel, as well as in a



FIG. III.

Irrigation canal overgrown with water weeds; Euphrates district, near Suk-esh-Shuyuk.

dry marsh. Empty shells of this important form were also obtained in canals at Ramadie, and were moreover abundant in a semi-fossil condition on the banks of the Tigris and the Euphrates in many localities (e.g. Amara, Zeur near Baghdad, and Felujah).

As mentioned above, my malacological work in Mesopotamia proved somewhat disappointing: much certainly remains to be done on the molluscan fauna of that country. The above section shows, however, that *Bullinus contortus* does occur in Mesopotamia, and, judging from the numerous semi-fossil specimens, must in fairly recent times have had a wide distribution. It is difficult to reconcile the rarity of its present occurrence with the comparatively frequent *Schistosoma haematobium* infections among the Arabs. Infection experiments should certainly be conducted on some of the other common Gastropoda of Lower Mesopotamia.

The question of the occurrence of *Schistosoma mansoni* in Mesopotamia has not been touched on in this Report, as I have had no opportunity of examining faeces from the Arab inhabitants. Only two cases of rectal bilharziasis came under my notice during my stay in the country; both of these proved to be due to *Schistosoma haematobium*, and the faeces showed the typical terminal-spined ova of that species. The abundance of *Planorbis* everywhere suggests that a suitable host exists in Mesopotamia to ensure the spread of *S. mansoni*.

In dealing with the Molluscs in the above section, no attempt has been made to give the specific names of the different forms. The collections have been sent to Dr. N. Annandale at the Indian Museum, Calcutta, who has kindly agreed to work them out in conjunction with other collections from Mesopotamia, Persia, and India.

### SUMMARY.

1. Only one outbreak of bilharziasis occurred among the troops in Mesopotamia up to August 1918, 71 of the Indian personnel of a General Hospital having become infected with *Schistosoma haematobium* at Basra during the summer of 1917.

2. The results of an investigation of the Arab population of Mesopotamia showed that the disease was common throughout the country, both in the Tigris and the Euphrates districts; the average infection by *Schistosoma haematobium* was approximately 20 per cent of the male Arabs examined.

3. The mollusc *Bullinus contortus*, known to be the second host of the parasite in Egypt, was found in Mesopotamia, but does not seem to be of common occurrence in that country.

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# COMA AS A CAUSE OF DEATH IN DIABETES.

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IN previous papers dealing with the work done during the last four years, in connection with the causes of the prevalence of diabetes in India, we have referred constantly to coma as a terminal phenomenon. From the very beginning of the enquiry we have been doubtful as to the accuracy of the usually accepted diagnosis that the coma, which not infrequently ends the scene, is diabetic. In the autumn of 1916 we had definitely arrived at the conclusion as was stated in the *Indian Medical Gazette*—that the coma seen in the type of diabetes met with in India is uræmic and not diabetic.

As case after case was studied and investigated and as practically no evidence of acidosis could be obtained, either during the course of

the disease or even when the patients were deep in coma, our suspicions became intensified. The evidence up to this time was mostly of a negative nature; in a few cases we had estimated the urea of the blood and found that the percentage was considerably increased. It was not, however, until early in 1917 that we began seriously to investigate the condition of the blood in order to acquire more complete data. At once positive evidence of the uræmic nature of the so-called diabetic coma was obtained.

In order to get our facts clear and convincing it was considered necessary to carry out a detailed investigation.

We therefore set about the analysis of the blood in practically all cases of diabetes, with or without albuminuria, all cases of kidney diseases with or without uræmia, and also in a large number of perfectly normal individuals. The literature of this subject is very meagre and in India difficult to obtain. Later, we were able to obtain some references to work done on the chemical examination of the blood in kidney disease and uræmia, but, so far as we know, no research has been carried out on the chemical condition of the blood in diabetes and diabetic coma, except as regards sugar, acetone and diacetic acid.

In the absence of any real signs of acidosis in the type of diabetes we were dealing with, and as, from clinical observations, we suspected uræmia rather than diabetic coma, we began the blood analysis with the object of seeking evidence of the retention of nitrogenous waste-products, and not with the expectation of finding marked signs of acidosis.

#### UREA RETENTION IN URÆMIA.

As already stated, in all severe cases of coma we had been accustomed to estimate the percentage of urea present, but we felt that this was insufficient, as not infrequently we came across cases of uræmia where the percentage of urea was lower than in others who showed little sign of uræmia. In connection with the varying urea content of the blood in different forms of kidney diseases it is necessary to bear in mind two factors, at least, that tend to mask the retention of urea. The first is the accompanying retention of fluids, and the other is the fact that the urea content of all organs and tissues is approximately uniform, and approximately equal to that of the blood both in normal conditions and when there is an abnormally large amount of urea present. Fatty tissue



and the tissues of the urinary tract are the only exceptions—fat contains very little whilst the urinary tract tissues are well supplied with urea. (Marshall and Davis: *Journal of Biological Chemistry*, Vol. XVIII). Reasoning on these lines it seemed very probable that there might be a very considerable retention of urea, particularly when œdema, anasarca, etc., were present, and yet the percentage present in the blood might not appear to be greatly increased. For instance, an average Indian weighs 50 to 60 kilos, and would have roughly  $3\frac{1}{2}$  to 4 litres of blood. He forms about 15 grammes of urea per day, so that failure to eliminate would show itself comparatively quickly if all the urea were retained in the blood. Since, however, urea is taken up uniformly by all tissues, the production of 15 grammes daily, even if only 10 grammes were being excreted, would mean only a very slight increase in the percentage of urea in the blood. This would be further decreased by any retention of fluids such as occurs prior to the onset of œdema, ascites, etc.

As it requires a concentration of about 1 per cent of urea in the blood to prove fatal, it is very improbable that urea is the only, or the most important, toxic agent in the causation of uræmia. It is considered probable that urea does act in the higher concentrations on the central nervous system, producing muscular twitchings, vomiting and convulsions. (Herter.)

So far as our analyses go, the highest figure we have obtained for the urea concentration was 0.45 per cent, which seemingly would not in itself account for the onset of a fatal uræmia. Some authors give figures up to 0.65 per cent and even higher, but we have met with nothing in our researches approaching these results.

It is generally accepted that any figure over 0.25 grm. per litre or 0.025 per cent is abnormal and that, when urea is present in the blood in a concentration of over 0.10 per cent, uræmic signs and symptoms may be expected.

The results of our observations on the urea content of the blood will be given along with the records of determinations of the other constituents, but, as the estimation of the urea alone appeared to be insufficient, we turned to the investigation of the blood for signs of retention of the other non-protein nitrogenous waste-products; at the same time as complete analysis as possible was carried out in order to get some light on the problem of the causation of coma in the Indian type of diabetes.

With this object in view the blood was analysed under the following headings :—

Total Nitrogen.	Acetone.
Non-protein Nitrogen.	Fat.
Urea.	Phosphates.
Sugar.	Chlorides.
Alkalinity.	

#### URIC ACID AND CREATININ RETENTION IN URÆMIA.

Myers with several co-workers, Fine Chace, Lough, lays great stress on the importance of the estimation of the uric acid and creatinin of the blood in early cases of nephritis.

Myers and Chace (*Journal of the American Medical Association*, 1916) state that normally creatinin is the most readily, and uric acid the least readily, eliminated by the kidney, with urea standing in an intermediate position. The excretion of uric acid would be the first to be impaired, then urea, and lastly, as a terminal event, creatinin. They state that the handicap of a high creatinin content the kidney is, apparently, never able to overcome. A rise, therefore, in the concentration of uric acid of the blood would appear to be an excellent early sign in chronic interstitial nephritis of retention of nitrogenous waste-products. So far as we can discover from the literature available in India, which is exceedingly limited, no one has made any investigation on the retention of uric acid or creatinin in diabetes and in coma. If, as stated above, uric acid retention is an early sign of renal trouble, from the result of our observations we should expect to find uric acid and creatinin as well as urea retained in those patients dying of so-called diabetic coma.

It is now well-recognised that different kidney conditions lead to the retention of different urinary constituents, the others being allowed to pass through the glomeruli and uriniferous tubules. Thus, water and salt or urea or any nitrogenous waste product, such as uric acid, creatinin, etc., or phosphates or sulphates may be held up separately and individually. We may therefore get the picture shown by analyses of the blood varying very greatly according to the particular set of constituents that is being retained. Thus, in diabetes complicated by renal disease which leads to œdema—a fairly common type—we should expect to find a retention of water and salts greatly predominating over any retention of urea : whereas, in those cases complicated by renal disease of the

chronic interstitial type, the non-protein nitrogenous retention is much more in evidence.

This may afford a suitable explanation of the blood findings in those cases where, although the urea percentage is not greatly increased, the other non-protein nitrogenous constituents are increased and the patients die with all the usual signs of uræmia.

#### RETENTION OF NON-PROTEIN NITROGENOUS WASTE- PRODUCTS IN URÆMIA.

Uræmia has been defined as an autotoxication by nitrogenous substances, depending on a disturbance of renal function, and accompanied by a decomposition of albumen (plasmolysis) leading to abnormal acid formation. (Nothnagel.)

A very considerable amount of investigation has been carried out with regard to the relationship between the onset of uræmia and the accumulation of nitrogenous waste-products in the blood.

Bang states that normally the average figure for non-protein nitrogen is 0.025 per cent, of which 0.015 is urea. According to Bang, the variations are slight and the ingestion of an average protein dietary does not lead to any marked increase in the non-protein nitrogen—the urea is slightly increased.

Van Slyke and Meyer (*Journal of Biological Chemistry*, Vol. XVI), in discussing the fate of the digestion products in the body, state that they have proved by the nitrous acid method that the amino-acids enter direct into the blood stream, from which they rapidly disappear as the blood circulates through the tissues. They conclude from their observations that amino-acids are intermediate steps not only in the synthesis but also in the breaking down of body-proteins, as they are not diminished but increased during starvation.

Autolysis is the main source of free amino-acids of the living body, and high protein feeding fails to increase the free amino-acid content of the blood.

On the other hand, Folin, Denis, Seymour, Mosenthal and Lewis lay stress on the important part diet plays in influencing the level of nitrogenous waste-products in the blood. With this opinion we are in agreement, if for no other reason than the evidence afforded by the very satisfactory lowering of the non-protein nitrogen that can be effected by a low protein dietary in cases of early uræmia. That is, whatever doubt there may be to regard the influence of high protein feeding in

causing an increase in the nitrogenous waste-products in health, there is no doubt at all that a patient on the verge of uræmia may have an attack precipitated by an excessive protein dietary, or may be saved from uræmic coma by protein starvation.

In carrying out our investigations on the non-protein nitrogenous constituents of the blood we considered it wise to eliminate the influence of diet as far as possible. This was done by arranging in all cases to take the blood for examination about the same time after the previous meal. In those cases where the patient was very seriously ill, the factor of diet hardly enters into the question.

Another point that must be taken into consideration in the estimation of the percentage of non-protein nitrogenous waste-products is the dilution, if any, of the blood that accompanies that retention. The great majority of the blood examinations so far made, to which we could refer, give the urea, uric acid, non-protein nitrogen, etc., in percentage without any reference to the specific gravity of the blood or to the degree of water retention that has taken place. This retention of fluids may mask completely the holding up of urinary constituents. Thus chlorides, for instance, may be very completely retained in the body and yet the percentage of chlorides in the blood may vary not at all or only to a slight extent. In order to overcome this fallacy in the analyses we decided to rely on the relationship between the total nitrogen and non-protein nitrogen of the blood. Any dilution of the blood will affect each of these constituents similarly and will not disturb the proportion that should exist between them.

Scheel (*Ugeskrift for Læger*, Copenhagen, No. 15) reports an extensive research on the retention of "rest" nitrogen in the blood as an index of renal function. He is convinced that determination of the residual nitrogen is as necessary a routine measure as, and even more important than, examining the urine for albumen. It differentiates real kidney disease from various conditions, such as heart disease and high blood pressure, which simulate it closely. It serves as a guide to treatment and throws light on the prognosis. Its importance cannot be exaggerated in treatment as it points out those cases of nephritis that may be given protein freely and those from whom it must be withheld.

He finds in nephritis with uræmia that the non-protein nitrogen is present in abnormal amounts from 0.10 per cent to 0.289 per cent.

In nephritis, mild without uræmia, the residual nitrogen is increased but slightly. A test meal may be necessary to determine the functional

capacity of the kidneys. A meal containing 85 grammes of protein has no effect in increasing the residual nitrogen in ordinary health, but if the kidneys are seriously affected the non-protein nitrogen may be doubled in a few hours.

Scheel says that a rise in the residual nitrogen of the blood may be the only sign of an impending uræmia. Although in itself it does not seem to be responsible for any specific symptoms, it is a very useful index of the toxæmia and its degree. Scheel also lays stress on the importance of estimating the proportion of water in the serum, as the dilution will affect the proportion of nitrogen and may mask the amount retained.

This is a most interesting paper from our standpoint, as Scheel's results bear out our own findings in almost every detail, as will be seen when we give our analyses of the blood in the different types of albuminuria. So far as we are aware this is the only publication that deals with this important subject in the same manner as we have tried to do. Unfortunately we have only been able to obtain a short summary of Scheel's paper, published by the *Journal of the American Medical Association*, 1916, but, so far as the results are available, they agree in a remarkable manner with our findings in similar kidney conditions. In our researches we have applied the same method of investigation to diabetes and the results are exceedingly interesting and important.

It may be accepted therefore that the upper limits of safety for the non-protein nitrogen of the blood, on an average diet, may be taken as about 0.03 per cent. Foster gives a maximum of 0.044 per cent; Scheel, 0.04 per cent; Denis, Folin, 0.025 per cent; so that anything over 0.03 per cent may be regarded as indicating some degree of renal insufficiency.

Regarding the onset of uræmia, a non-protein nitrogenous content of 0.09 per cent or a high urea nitrogen of 0.065 per cent (0.13 per cent urea) or higher, has been found to be the most reliable prognostic sign.

As already pointed out, it is a much safer procedure to base our judgment on the proportion of non-protein nitrogen to the total nitrogen of the blood, rather than on actual percentages. The retention of water may go far to mask the inefficiency of the kidneys in excreting the toxic nitrogenous waste-products.

One more point may be referred to with regard to uræmia. It is well recognised that the picture presented by uræmic patients varies in the most bizarre fashion. The clinical manifestations are manifold and various, and seldom are two cases found exactly similar.

Foster (*Journal of the American Medical Association*, 1916), in a very interesting article, discusses the differentiation of the several types of uræmia and arranges them into three classes :—

- (1) Retention type : Urinary poisoning of Ascoli. This is characterised by anuria or relative suppression of the urine. Convulsive seizures and nervous phenomena are absent, gastro-intestinal troubles are rarely present, or only as a terminal phenomenon. A gradually deepening coma is the common condition, progressive asthenia and anorexia followed by stupor and death.
- (2) Cerebral oedema type : this is the condition associated with large white kidney, in which there is depression of the power of excreting water and salts with a relatively normal excretion of urea and other normal nitrogenous fractions.

This form of nephritis, if pure, is seldom associated with uræmia : sometimes, however, uræmic symptoms develop, such as vomiting, headache, stupor, amaurosis and coma. The blood picture remains fairly normal, there are only slight signs of nitrogen retention even when the excess water present is taken into consideration. He suggests that this type is due to cerebral oedema.

- (3) Toxic type or epileptiform uræmia is regarded as peculiar and specific and entirely different in causation from the other types of uræmia. Foster states that he has isolated a crystalline body from the blood which is the cause of the toxicity and the convulsions.

In this type there is an increase in the non-protein nitrogen of the blood and he suggests that an abnormal nitrogenous compound, incapable of being excreted, is formed. This crystalline substance on injection into an animal causes dyspnœa, muscular twitchings, convulsions, coma and death. The substance is the result of an abnormal nitrogenous katabolism with the consequent formation of toxic bodies which cause uræmia.

It may be accepted that certain forms of uræmia show a retention of nitrogenous waste-products. In some forms the retention of non-protein nitrogen or of urea nitrogen may not be very conspicuous or the non-protein nitrogen may be retained out of all normal proportion to the



urea. The types Foster describes are never pure and in every case. Scheel and our own results prove, uræmia is accompanied, if not caused, by a gross retention of the non-coagulable albuminous constituents of the blood.

These results have been very generally accepted as accompanying kidney diseases and uræmia. So far as we are aware, however, no observations have been made regarding the condition of the blood, except from the acidosis standpoint, in diabetes and diabetic coma.

Albuminuria is of course a very common complication of glycosuria, and the close relationship of nephritis to diabetes has not hitherto received sufficient attention. True nephritis, usually of the granular type, is very common. Sometimes albuminuria may precede or accompany the sugar, often the two conditions, albuminuria and glycosuria, may alternate. As the nephritis gets worse the glycosuria lessens, but this is usually a sign of bad omen. Other renal complications have been described by various authors but have not been looked upon as greatly enhancing the death-dealing power of diabetes.

Thus, "simple diabetic albuminuria is a benign process, often transient, and stands in no known relation with true nephritis" (Allan). It is supposed to be caused by irritation of the kidney by the glycosuria.

Our investigations on the blood would certainly point to the simple diabetic albuminuria not perhaps being so benign as it appears. In the mild type of diabetes common in India, where acidosis is practically unknown and coma of a diabetic nature very rarely seen, the disease lasts long and the simple benign albuminuria assumes a very much more menacing appearance than the general opinion of its significance would warrant.

In our opinion it takes the place of so-called acidosis in the diabetes of the more severe types. Both often end in coma, the mild Indian variety in uræmic coma, the more severe diabetes of temperate climes in diabetic coma.

It would be most interesting to have a series of blood analyses carried out in cases of pure diabetic coma—those which show marked signs of acidosis. So far we have not had sufficient opportunity of analysing the blood in typical cases of diabetic coma, so we are unable to speak definitely on the subject. We should, however, be very much surprised if analyses did not show that even in the present forms of acidosis and diabetic coma, there is a marked retention of nitrogenous waste products, and that a very large part of the coma is uræmic in



nature. Since this was written, the opportunity has come and the results—case 162—bear out our prophecy.

From the evidence we shall now proceed to bring forward we have no hesitation in stating that the coma ending the scene in the Indian type of diabetes is uræmic and not diabetic.

In connection with this part of the investigation, between four and five hundred blood analyses have been carried out. We shall classify our results under the following headings:—

- I. Normal healthy individuals.
- II. Glycosurics: no albuminuria.
- III. Mild albuminuria: no glycosuria.
- IV. „ „ : glycosuria.
- V. Severe kidney cases: no glycosuria.
- VI. Glycosurics dying in coma or very seriously ill.

At the end of the table showing the result of the blood analyses in severe or dying cases of diabetes, we shall give the analyses of the blood in two severe cases of diabetes gravis complicated by all the usual signs of acidosis, which is described as commonly met with, as a terminal phenomenon, in the severer form of the disease prevalent in temperate climates. One of these cases passed acetone and diacetic acid for over a year before succumbing to diabetic coma: the other was diabetes of acute onset, marked early acidosis, coma, pneumonia, septic parotitis, followed by recovery.

TABLE I.  
*The Chemical Composition of the Blood in Health.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N. P. N. T. N.	REMARKS.
		%	%	%	%	%	%	%	%		
1	Normal ..	3.50	.025	.03	.086	.02	.55	$\frac{n}{32.5}$	.18	$\frac{l}{140}$	
2	Hydrocele ..	2.80	.028	.035	..	..	..	..	..	$\frac{l}{100}$	
3	Glands ..	2.90	.028	.035	.078	..	..	..	.14	$\frac{l}{104}$	

*Coma as a Cause of Death in Diabetes.*TABLE I—*Contd.*

No.	Case	Total N trogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlo. es.	Alkalinity.	Fat.	N. P. N T. N.	REMARKS
		<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>	<sup>g</sup> / <sub>100</sub>		
4	Normal	3.20	.028	.04	.096	..	..	..	..	$\frac{1}{114}$	
5	Tumour	3.15	.032	.04	.12	..	..	..	..	$\frac{1}{99}$	
6	Glands	3.30	.0238	.04	.096	..	..	..	.10	$\frac{1}{138}$	
7	Asthma	3.26	.026	.04	.13	..	..	..	..	$\frac{1}{125}$	
8	Injury	2.24	.016	.03	..	..	..	..	..	$\frac{1}{140}$	
9	Syphilis	1.66	.0168	.04	..	..	..	..	..	$\frac{1}{100}$	
10	Tumour	2.94	.029	.023	..	..	..	..	..	$\frac{1}{100}$	
11	Tumour	3.03	.021	.032	.116	..	..	..	.10	$\frac{1}{143}$	
12	Normal	2.95	.019	.03	.12	.0175	.575	$\frac{n}{30}$	.08	$\frac{1}{155}$	
13	Fracture	2.38	.021	.038	.20	.015	.590	$\frac{n}{30}$	.20	$\frac{1}{114}$	Died.
14	Normal	3.09	.021	..	..	..	..	..	..	$\frac{1}{147}$	
15	Healthy	2.82	.032	.03	.086	.02	.575	$\frac{n}{30}$	..	$\frac{1}{90}$	Inflamed ear.
16	Tumour	3.08	.025	.04	.096	.015	..	$\frac{n}{30}$	.04	$\frac{1}{123}$	European.
17	Normal	3.03	.03	.039	.096	.015	..	$\frac{n}{30}$	..	$\frac{1}{101}$	Do.
18	Skin	2.88	.02	.034	.107	.03	.65	$\frac{n}{35}$	.08	$\frac{1}{114}$	
19	Abscess healing	2.67	.018	.025	.156	..	..	..	.12	$\frac{1}{148}$	
20	Alcohol	2.81	.021	.025	.16	.06	..	$\frac{n}{37.5}$	.08	$\frac{1}{106}$	

TABLE I.—*Concluded.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%	%	%		
21	Infantilism ..	3.09	.023	.04	.169	.03	.67	n 30	.24	$\frac{1}{134}$	
22	Ditto ..	3.20	.021	.035	.179	.015	.625	n 35	..	$\frac{1}{152}$	
23	Ankylostoma ..	2.28	.022	.04	.076	.025	.518	n 40	.20	$\frac{1}{100}$	
24	Weak heart ..	2.62	.017	.024	.13	..	..	..	.12	$\frac{1}{154}$	
25	Normal ..	2.68	.024	.03	.09	..	..	..	..	$\frac{1}{112}$	

These twenty-five practically normal individuals, so far as any retention of waste-products in the blood is concerned, give a good idea of the percentages of the different constituents present. We are specially concerned with the proportion of non-protein nitrogen to the total nitrogen. The variations met with in health are between  $\frac{1}{100}$  to  $\frac{1}{100}$  or even higher. The urea of the blood is rarely higher than 0.04 per cent. (urease method). The phosphates average 0.025, chlorides 0.565, and the alkalinity  $\frac{n}{32.5}$ .

We shall now collect together those cases of simple glycosuria where otherwise the patients appeared healthy and gave no signs of albuminuria or kidney trouble. So far as hospital practice is concerned, this type of case is comparatively rare. The simple albuminuria which commonly accompanies glycosuria is an early complication of diabetes in India, and, as the blood analyses will show, this albuminuria in its turn is nearly always accompanied by a retention of nitrogenous waste-products.

## Coma as a Cause of Death in Diabetes.

TABLE II.

*Mild Diabetes: No Albuminuria.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%		%		
26	Diabetic (improved greatly by treatment).	2.67	.016	.048	.58	..	..	..	.14	$\frac{1}{167}$	No albuminuria. { The glycosuria disappeared and the carbohydrate tolerance was re-established.
		3.15	.0305	.043	.17	..	..	..	..	$\frac{1}{103}$	
		2.97	.024	.031	.18	.02	.575	$\frac{n}{30}$	.08	$\frac{1}{123}$	
27	Old diabetic ..	3.34	.021	.035	.22	..	..	..	..	$\frac{1}{159}$	No albuminuria. Did very well.
		3.26	.026	.045	.145	..	..	..	..	$\frac{1}{125}$	
28	Diabetic ..	3.48	.03	.035	.45	..	..	..	.20	$\frac{1}{112}$	Early case. Did well under treatment.
29	Old diabetic ..	3.36	.042	.04	.17	..	..	..	.12	$\frac{1}{80}$	Convalescent. Did well under treatment.
30	Robust diabetic.	4.30	.042	.04	.22	..	..	..	.12	$\frac{1}{102}$	Severe glycosuria. Did very well under treatment.
31	Old diabetic ..	4.20	.042	.04	.096	..	..	..	.12	$\frac{1}{100}$	Convalescent. Severe neuritis. Cured and carbohydrate tolerance re-established.
		4.20	.042	.03	.096	..	..	..	.16	$\frac{1}{100}$	
		3.85	.042	.04	.128	..	..	..	.10	$\frac{1}{92}$	
32	Old diabetes, carbuncle.	3.52	.042	.06	.46	..	..	..	.14	$\frac{1}{84}$	Did well eventually.
33	Cured diabetes	2.95	.019	.035	.12	.017	.58	$\frac{n}{30}$	.08	$\frac{1}{155}$	History of diabetes, otherwise healthy.
34	Diabetes; very fat.	3.05	.021	.04	.13	.02	..	$\frac{n}{32.5}$	..	$\frac{1}{145}$	Convalescent. Improving under treatment.
35	Diabetic anaemia.	2.60	.027	.038	.35	..	..	..	.64	$\frac{1}{99}$	Sugar of urine 6.50%; acetone, nil.

These ten cases are sufficient for our purpose, which is to show that the presence of hyperglycæmia or glycosuria need not be accompanied by any signs of retention of nitrogenous waste-products. The figures for the percentages of the blood constituents shown by these ten cases run fairly well parallel to those given for normal individuals. It is difficult to collect many cases under the heading of mild diabetes: no albuminuria. These ten are all we possess out of hundreds of analyses. The reason is, of course, that albuminuria is an early and almost constant complication of the type of diabetes prevalent in India. Another reason is that patients with glycosuria do not consider themselves seriously ill until albuminuria sets in: they then come to hospital or seek skilled advice.

The tendency, however, even when the clinical tests for albuminuria are absent, is for the non-protein nitrogen of the blood to be on a higher plane. Doubtless as sugar is being eliminated the processes that lead to albuminuria are already at work and the kidney function is already becoming impaired.

We shall now take up the next class of case, *viz.*, those where the gross sign of albuminuria is evidence that the kidney function is not normal. Table III presents those of this type where there is no diabetes: Table IV those where there is diabetes.

TABLE III.

*The Chemical Composition of the Blood in mild Albuminuria: No Glycosuria.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.		Phosphates.		Chlorides.	Alkalinity.	Fat.	N.P.N T.N.	REMARKS.
		%	%	%	%	%	%	%	%			
36	Kala-azar, œdema.	3.124	.042	.042	.096	.015	.612	$\frac{n}{30}$	.14	1	74	Albuminuria ++
37	Nephritis of pregnancy.	2.80	.031	.050	.128	.025	..	$\frac{n}{30}$	..	1	90	Convalescent.
38	Nephritis, no casts.	1.599	.05	..	..	..	..	..	..	1	32	Very anæmic and œdematous.

TABLE III.—Contd.

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	°/o	%	%	%	%	%	%		
39	Nephritis, anasarca.	2.62	.18	.050	.15	..	..	..	.128	$\frac{1}{14}$	Urine : albumen .1°/o. Acetone, . . . nil. Blood : acetone nil.
	Do. (12 days later).	2.24	.105	.025	.156	.02	..	n 32.5	..	$\frac{1}{21}$	
40	Nephritis ..	2.10	.077	.035	.169	.015	..	n 40	..	$\frac{1}{27}$	Urine : albumen .2°/o. Casts + + No acetone in blood or urine.
41	Nephritis, oedema.	3.24	.056	.030	.107	.015	..	n 30	.04	$\frac{1}{58}$	No acetone in blood.
42	Do.	2.86	.10	.038	.12	..	..	..	.22	$\frac{1}{29}$	Albuminuria .025°/o.
43	Do	3.024	.06	.025	.086	.015	..	n 32.5	.38	$\frac{1}{50}$	Do. .30°/o. Acetone, . . . nil.
44	Do	1.97	.073	.060	.156	..	..	..	..	$\frac{1}{27}$	Albuminuria .17°/o. Casts + + +
	A week later. .	2.08	.042	.063	.146	.025	.512	n 38.5	.28	$\frac{1}{50}$	Very ill but rallied and improved.
Serum drawn from the legs contained .04°/o urea.											
45	Empyemia ..	2.24	.021	..	.116	.04	.525	n 35	.20	$\frac{1}{106}$	Urine : albumen .02°/o. Nucleoprotein + +
46	Surgical kidney.	2.80	.088	.068	.156	.02	.65	n 37.5	..	$\frac{1}{32}$	Old man : operation on bladder. Died.
47	Nephritis, anasarca.	2.52	.098	.039	.12	..	..	..	.12	$\frac{1}{26}$	Urine : albumen 0.75°/o.
48	Do. ..	2.21	.113	.07	.116	..	..	..	.08	$\frac{1}{20}$	Do. Do. .025°/o.
49	Liver abscess ..	2.89	.056	.042	.087	.025	.625	n 35	..	$\frac{1}{51}$	(Do. Do. .025°/o. No casts.
50	Pernicious anemia.	1.68	.049	.042	.107	.02	.525	n 35	.28	$\frac{1}{34}$	Albuminuria : Oedematous, very anemic.
51	Ascites ..	1.87	.03	.038	.16	..	..	..	.16	$\frac{1}{62}$	Albuminuria, a trace.
52	Anemia, anasarca.	1.68	.03	.03	.19	..	..	..	.08	$\frac{1}{56}$	Do. Do.
53	Ascites, oedema.	3.15	.10	.042	.086	.02	.612	n 30	..	$\frac{1}{31}$	Do. Do.

TABLE III.—*Contd.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%	%	%		
54	Beri-beri, œdema.	1.68	.028	.025	.096	..	..	..	.16	$\frac{1}{60}$	Urine : albumen .075%. Casts + +
55	Nephritis, œdema.	2.53	.098	.04	.179	..	..	..	..	$\frac{1}{26}$	Urine : albumen + +.
56	Do. ..	2.30	.096	.045	.13	..	..	..	.24	$\frac{1}{24}$	Do. Do.
57	Do. ..	1.624	.085	.045	.12	..	..	..	.12	$\frac{1}{20}$	Do. Do.
58	Do. ..	1.85	.10	..	..	..	..	..	..	$\frac{1}{18.5}$	{ Urine: albumen .3%. Casts + + +
59	Do. plus ascites.	2.73	.0518	.038	.12	.015	.60	n 30	..	$\frac{1}{53}$	
60	Beri-beri ..	2.35	.09	.060	..	..	..	..	.12	$\frac{1}{26}$	Urine : albumen .075%. Œdematous.
61	Do. ..	1.84	.026	.028	.09	..	..	..	.09	$\frac{1}{66}$	Urine : albumen .015.
62	Do. ..	2.66	.066	.025	.179	..	..	..	.28	$\frac{1}{40}$	Do. Do. 0.15%.
63	Nephritis, anasarca.	1.838	.056	.028	..	..	..	..	..	$\frac{1}{3}$	Urine: albumen .25%
64	Nephritis, asthma.	2.81	.10	.03	.078	.025	.625	n 28.5	..	$\frac{1}{28}$	Urine : albumen and casts.
65	Nephritis, anæmia.	2.18	.112	.03	.128	.015	.625	n 30	..	$\frac{1}{20}$	Urine : albumen ! +
66	Cancer : jaw ..	2.59	.076	.065	.116	..	..	..	.14	$\frac{1}{34}$	{ Urine: albumen .2%. Casts present.
67	Tumour ..	3.44	.048	.03	.11	..	..	..	.08	$\frac{1}{72}$	Urine : albumen, a trace.
68	Hernia ..	3.35	.058	.037	.116	.04	.525	n 30	.18	$\frac{1}{58}$	Do. Do.
69	Hydrocele ..	3.21	.148	.05	.116	..	..	..	..	$\frac{1}{22}$	Urine : albumen + + +



TABLE III.—*Concl'd.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%	n 30	%		
70	Interstitial nephritis.	3.16	.09	.048	.096	.025	.65	n 30	..	$\frac{1}{35}$	Urine : albumen + + Casts + + + Blood pressure 145 m.m.
	(8 months later)	3.37	.14	.058	.13	..	..	..	.12	$\frac{1}{24}$	Do. do.
	(6 months later)	3.22	.112	.038	..	..	..	..	..	$\frac{1}{28}$	Do. do.
71	Nephritis ..	2.24	.07	.078	.096	..	..	..	..	$\frac{1}{32}$	Albuminuria.
72	Kala-azar, oedema.	1.96	.039	.03	.13	.015	..	n 30	..	$\frac{1}{50}$	Do.
73	Nephritis, ascites.	1.56	.126	.04	.156	..	..	..	.08	$\frac{1}{12}$	Urine : albumen 3% Casts + + +
74	Anasarca, anaemia.	2.88	.053	.04	.079	..	..	..	.08	$\frac{1}{54}$	Urine : albumen, a trace.
75	Nephritis, oedema.	2.80	.10	.079	.16	..	..	..	.16	$\frac{1}{28}$	Urine : albumen + + + Casts + +
76	Nephritis, asthma.	3.37	.12	.038	.13	..	..	..	.08	$\frac{1}{28}$	Do. do.
77	Meningitis, coma.	2.814	.06	.042	.18	.02	.612	n 35	.16	$\frac{1}{47}$	Urine : no record.
78	Coma ?	2.38	.054	.04	.13	..	..	..	.28	$\frac{1}{44}$	Do. do.
79	Heart plus kidney.	2.96	.196	.04	.146	.02	.625	n 30	.08	$\frac{1}{15}$	Urine : albumen + + +
80	Nephritis, anasarca.	2.39	.063	.037	.116	.015	.612	n 30	..	$\frac{1}{38}$	Urine : albumen + +
	(later)	2.32	.071	.042	.116	.015	.612	n 30	..	$\frac{1}{33}$	

These forty-five cases of albuminuria, in varying degrees, are sufficient to demonstrate the very great tendency to non-protein nitrogen retention that takes place as soon as the kidney becomes affected. As soon as albuminuria sets in, the ratio  $\frac{\text{N.P.N.}}{\text{T.N.}}$  at once tends to rise from 1 : 100 or 1 : 150 to a much higher proportion.

As will be seen, the urea nitrogen of the blood may be no criterion at all of the degree to which the other nitrogenous waste-products (or nitrogenous bodies abnormally formed) may accumulate in the blood.

The greater the kidney involvement, the more readily the retention takes place and the higher the ratio of non-protein nitrogen to the total nitrogen of the blood.

We have failed to find any marked increase in the phosphates of the blood, as has been said to occur in these cases showing a tendency to uræmia. Similarly, in renal cases of the degree of severity that obtained amongst the forty-five included in the above table, we have not found any large measure of departure from the normal figure for the alkalinity of the blood.

TABLE IV.

*The Chemical Composition of the Blood when both Glycosuria and Albuminuria are present.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.		Sugar.		Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	‰	‰	‰	‰	‰	‰	‰	‰	
81	Diabetic.	2.85	.03	.035	.30	..	..	..	..	..	.10	$\frac{1}{95}$	Albuminuria and albumose, present.
	Gangrene and alcoholism.	2.97	.045	.035	.156	.05	.525	n	32	..	.12	$\frac{1}{66}$	
	No glycosuria despite hyperglycæmia.	3.09	.068	.053	.096	.03	..	n	35	..	..	$\frac{11}{45}$	Urea 1.6%
		2.32	.024	..	..	..	..	..	..	..	..	$\frac{1}{97}$	No improvement in gangrene until the blood improved, and albuminuria disappeared.
82	Old diabetic, septic keloid of chest.	2.99	.031	.035	.35	.015	..	n	30	..	..	$\frac{1}{92}$	Very stout and un-healthy.
	Two years' history.	3.22	.07	.025	.169	.01	..	n	30	.12	..	$\frac{1}{46}$	Glycosuria 2.20% Albuminuria.
83	European, diabetic, very fat.	3.18	.063	.035	.36	.015	..	n	35	.04	..	$\frac{1}{50}$	Albuminuria ++ Glycosuria 3.50%
84	Do. ..	3.02	.050	.045	.32	..	..	..	..	..	..	$\frac{1}{60}$	Albuminuria ++ Glycosuria 2.50%



TABLE IV.—Contd.

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%		%		
92	Diabetic, very ill.	3.22	.07	.10	.39	..	..	..	..	1 46	Glycosuria 8%.
		3.04	.056	.06	.169	..	..	..	.12	1 54	Albuminuria.
		3.38	.07	.08	.19	..	..	..	..	1 48	Toxæmic.
		3.65	.0338	.06	.169	..	..	..	..	1 108	Improved in every way and the carbohydrate tolerance re-established.
		3.68	.049	.06	.15	..	..	..	..	1 75	
		3½ months later, returned.	3.63	.084	.04	.22	.01	..	..	.08	1 43
93	Old diabetic, oedema.	2.04	.152	.038	.29	..	..	..	.04	1 14	(Albuminuria .1%.) (Glycosuria .8%.)
94	European female; diabetic, very ill.	3.34	.116	.10	.30	..	..	..	.16	1 29	Three large septic carbuncles, very toxæmic.
		2.88	.117	.04	.263	..	..	..	.16	1 19.5	Albuminuria, uræmic.
		2.67	.039	.04	.128	.015	..	n 30	.14	1 70	Improved greatly and albumen and sugar disappeared.
95	Young diabetic.	1.82	.028	.038	.39	..	..	..	.24	1 65	(Albuminuria + +) (Glycosuria 4%.) (Acetone nil.)
96	Acute diabetes, very ill.	Was excreting 30 pints of urine daily.									
		2.61	.12	.04	.33	..	..	..	.08	1 22	(Albuminuria + +) (Glycosuria 7%.)
		2.75	.20	.28	.22	..	..	..	.08	1 14	Recovered completely and carbohydrate tolerance re-established.
		2.82	.098	.03	.15	..	..	..	.08	1 30	
97	Diabetic: huge huge carbuncle on back.	The blood showed the presence of acetone.									
		3.01	.28	.08	.146	.02	.543	n 35	.28	1 11	Very ill: no glycosuria, septic and toxæmic.
		2.70	.050	.067	.130	..	..	..	.28	1 54	Improved rapidly, no sugar, albumen or acetone.



Many of these cases, from an ordinary examination of the urine, would not be considered as being in a serious condition. That is, the albuminuria in the majority was trifling and in few were there any casts or other signs of kidney trouble. Yet, the blood analyses show that the early signs of serious retention of nitrogenous waste-products had set in, which, if not checked, would eventually lead to cachexia, toxæmia, uræmia, and death.

An important point brought out by the records is the favourable influence exerted by anti-diabetic treatment on the elimination of these retained nitrogenous bodies : *vide* Cases 81, 86, 91, 92, 94, 96, and 97.

We shall now give the results of the analyses of the blood in those cases where the patients were dying or very seriously ill. Table V shows those who appeared to be pure kidney disease; Table VI, those who were diabetic first but who were really dying of uræmia, although the clinical diagnosis in the great majority would have been diabetic coma.

TABLE V.

*The Blood in Uræmia.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T. N.	REMARKS.
		%	%	%	%	%	%		%		
108	Kidney, anasarca	2.39	.063	.037	.116	.0075	.612	$\frac{n}{30}$	..	$\frac{1}{38}$	Very low : anæmic, chronic diarrhœa.
		2.32	.071	.042	.116	.015	.612	$\frac{n}{30}$	..	$\frac{1}{32}$	
		2.15	.30	.355	.13	.045	..	$\frac{n}{60}$	..	$\frac{1}{7}$	
109	Jaundice, chol- æmia, semi- coma to s.c. He only lived four days.	2.10	.298	.446	..	.035	..	$\frac{n}{45}$	..	$\frac{1}{7}$	Albuminuria + + Acetone <i>mit.</i> No acetone in the blood.
		2.14	.296	.437	..	.045	..	$\frac{n}{45}$	..	$\frac{1}{7}$	Ammonia of blood ".004%.
								$\frac{n}{45}$	..	$\frac{1}{7}$	The blood serum showed bile each day.
		1.96	.298	.454	..	.025	..	$\frac{n}{35}$	..	$\frac{1}{6}$	
										Died	

This patient was seemingly dying of cholæmia, yet the blood picture is that of uræmia. The albuminuria was slight.





TABLE V:-- *Contd.*[illegible]

## Coma as a Cause of Death in Diabetes.

TABLE V.—Contd.

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	$\frac{\text{N.P.N.}}{\text{T.N.}}$	REMARKS.
		%	%	%	%	%	%		%		
120	Prostate. Septic kidney, operation.	2.24	.112	.041	.116	.081	.525	$\frac{n}{30}$	.08	$\frac{1}{16}$	Suprapubic drainage, Urine: albumen, pus, blood.
The patient was very toxæmic and uræmic, but later began to improve and slowly recovered.											
121	Uræmia, dyspnoea.	2.56	.28	.17	.128	.025	.625	$\frac{n}{40}$	.22	$\frac{1}{9}$	Albuminuria copious.
										Died at once.	
122	Gangrenous appendix (moribund).	2.20	.33	.038	.107	..	..	..	.48	$\frac{1}{6.6}$	Albuminuria ++
										Blood serum showed oil globules.	
123	Eclampsia. Bl. pr. 130. m. m.	2.52	.113	.15	.20	..	..	..	.28	$\frac{1}{29}$	Died same day.
124	Cholemia, jaundice.	2.156	.156	.24	.16	..	..	..	.20	$\frac{1}{14}$	Muttering delirium.
										Died next day.	Urine: not obtained.
125	Uræmia. Bl. pr. 210 m. m. Hemiplegia developed. (Lived five days.)	3.08	.168	.07	.13	..	..	..	.12	$\frac{1}{18}$	Albuminuria ++ no casts, no acetone in blood or urine.
		3.08	.170	.06	.116	..	..	..	..	$\frac{1}{17}$	
		3.01	.168	.06	..	..	..	..	..	$\frac{1}{17}$	
		3.10	.28	.06	..	..	..	..	..	$\frac{1}{11}$	
										Amino-acids of blood .008%	Died in coma.
Blood after four days' anuria.											
126	Anuria. Hydro-nephrosis of right kidney; removed	3.28	.092	.163	..	..	..	..	..	$\frac{1}{35}$	
Eight days later, after drainage of left kidney.											
	Later, left ureter blocked for four days.	..	.102	.053	..	.03	.529	$\frac{n}{30}$	..	..	
127	Uræmia ..	3.12	.123	.15	..	..	..	..	..	$\frac{1}{25}$	Death.

TABLE V.—*Contd.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Sugar.	Phosphates.	Chlorides.	Alkalinity.	Fat.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%		%		
128	Uræmia ..	3.50	.126	.21	.21	..	..	..	.12	$\frac{1}{27}$	Death.
129	Eclampsia ..	2.17	.12	.09	..	[No acetone in blood.] ..				$\frac{1}{18}$	Do.
130	Do. ..	2.94	.112	.06	..	{ [Acetone present in blood ] [Amino-acids of blood 03%] }				$\frac{1}{25}$	Do. Albuminuria 1%. Acetone + +
131	Acute nephritis {	2.52	.084	.05	.16	{ [Amino-acids of blood .004] }				.2 $\frac{1}{30}$	{ Albuminuria .4%. Casts abundant.
	Edema, legs drained.	2.84	.110	.065	..	[No acetone in blood]				$\frac{1}{26}$	
	Serum from legs gave .075% of urea, i.e., greater than in blood.										
132	Acute nephritis, suppression for three days.	3.228	.148	.26	..	.05	..	n 50	..	$\frac{1}{22}$	{ Admitted semi- comatose and very cedematous. Reacted to treat- ment and eventu- ally recovered.
		3.22	.162	Next day. .335	..	.06	..	n 37.5	..	$\frac{1}{20}$	
		Amino-acids of blood .032%									
133	Kidney, liver abscess.	2.50	.18 No acetone in blood or urine.	.075	.128	.02	..	n 30	..	$\frac{1}{14}$ Died.	Albuminuria. Bile in blood.

The records of these twenty-six cases of severe nephritis, uræmia, etc., are of the greatest interest and afford considerable material for thought.

In every one the non-protein nitrogen content of the blood is greatly increased and the ratio  $\frac{\text{Non-protein nitrogen}}{\text{Total nitrogen}}$  radically changed.

The retention of urea, on the other hand, does not always run parallel to that of non-protein nitrogen. Evidently in certain types of uræmia urea is greatly held up: cases Nos. 109, 112, 114, 115, 121, 123, 124, 126, 127, 128, 132, are good examples of this. The details of the condition of the patients are insufficient to speak dogmatically, but it may be accepted that where there is marked œdema and anasarca

it is not usual to find great increases in the urea content of the blood.

Then, again, with regard to the phosphates and alkalinity of the blood, there would appear to be a certain degree of parallelism; where the phosphate content of the blood is high the tendency is for the alkalinity to fall—cases Nos. 109 and 112—but the variations are too great to afford grounds for definite conclusions.

Again the presence or absence of acetone in the blood does not appear to be a matter of fundamental importance in uræmia.

In connection with the condition of the blood in uræmia a number of analyses were made in severe cases of cholera to see if any light would be thrown on the cause of death. For this purpose we collaborated with Captain J. A. Shorten, I.M.S., who is responsible for the estimations of the phosphates, chlorides and alkalinity.

TABLE V. *Contd.*

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Phosphates.	Chlorides.	Alkalinity.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%			
134	Fatal cholera	..	..	·105	..	..	n 90		
		2·89	·177	·15	..	..	n 90	1 16	Fatal uræmia and marked decrease in alkalinity. Phosphates high, and great retention of non-protein nitrogen.
		2·87	..	·34	·125	..	n 95	1 10 ? Died.	
135	Do.	3·06	·106	·135	·04	..	n 40	1 30	Clinically death was due to collapse.
		3·85	·148	·05	·045	..	n 60	1 26 Died.	
		3·30	·098	..	·05	..	n 50	1 34	
136	Cholera (recovery)	2·715	·196	·14	·0625	..	n 35	1 14	
		2·81	·159	·116	·075	..	n 20	1 18	
		..	·051	·085	·04	..	n 37	1 60 ? Recovered.	

TABLE V. -contd.

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Phosphates.	Chlorides.	Alkalinity.	N.P.N. T.N.	REMARKS.
		%	%	%	%	%	%		
137	Cholera (recovery)	3.40	.056	.142	.05	.60	n 45	1 62	After being in a very serious condition for several days this patient gradually recovered.
		3.03	.163	.196	.03	.575	n 37.5	1 19	
		..	.169	.22	.03	.590	n 40	1 20	
		3.45	.169	.225	.025	.612	n 37.5	1 21	
		3.20	.182	.255	.015	.562	n 29.5	1 17	
		Two days later	2.98	.084	.05	.010	.547	n 28.75	
138	Cholera (recovery)	3.79	.07	.065	.125	.60	n 47.5	1 54	After severe collapse the patient improved.
		3.65	.084	.092	.05	.65	n 32.5	1 43	
		3.22	.169	.175	.075	.58	n 35	1 19	
							Recovered.		
139	Do.	2.28	.086	.060	.0175	.61	n 30	1 26	Fairly mild case.
		2.24	.070	.05	.0175	.617	n 28.5	1 32	
								Recovered.	
140	Do.	3.79	.056	.083	.075	.60	n 70	1 68	Do.
		3.40	.084	.038	.050	.66	n 60	1 40	
		3.22	..	.16	.020	.60	n 27.5	1 ?	
							Recovered.		
141	Fatal cholera	1.4	?	.17	..	Aminoacids .008%	..	Died	Had 16 pints of saline Three pints one hour before the blood was taken.
142	Do.	2.40	.112	.20	.04	..	..	1 21	Muttering delirium.
						Aminoacids .011%		Died.	

## Coma as a Cause of Death in Diabetes.

TABLE V—*concluded*.

No.	Case.	Total Nitrogen.	Non-protein Nitrogen.	Urea.			Alkalinity.	N.P.N. T.N.	REMARKS.
				%	%	%			
143	Fatal Cholera	.. 3.30	.09	.075	..	..	..	1 37 Before transfusion. Died later.	
					Aminoacids	.010%			
144	Do.	.. 2.54	.122	.26	.03	..	n 49	1 20 Died.	Toxæmic and incoherent.
145	Do.	.. 2.80	.142	.185	.045	..	n 50	1 20 Died two hours later.	
146	Do.	.. 2.40	.126	.160	.05	..	n 60	1 19 Died.	
147	(Recovery)	.. { 2.71	.14	.09	.035	..	..	1 19	
		..	.09	.03	..	..	n 35	Recovered.	

These cases of cholera, most of which were accompanied by more or less degree of suppression of urine, show the typical blood picture of uræmia, agreeing in their main characteristics with those tabulated in the earlier numbers of this class (108 to 133). The relationship  $\frac{\text{Non protein Nitrogen}}{\text{Total Nitrogen}}$  would appear to be the most reliable guide to the condition of the patient and as a basis for prognosis.

Of course in cholera there are probably other poisons at work besides ordinarily retained nitrogenous bye-products, and the fact that the poisons due to the cholera bacillus are not being eliminated would increase the gravity of the prognosis, apart from the degree of non-protein nitrogenous retention.

In choleraic uræmia the percentages of the phosphates and the alkalinity of the blood tend to depart further from normal standards than was found to be the case in ordinary uræmia from kidney disease.

Marriott and Hæssler believe that the retention of acid phosphates is sufficient to account for the degree of acidosis observed in nephritis. They state that this retention of acid phosphate in nephritis is not part of a general salt retention; that it seems to be due to a certain 'specificity' of retention because there was no corresponding increase of sodium chloride with the increase in acid phosphate: neither is it proportional to the total nitrogen and the urea retention in these cases. They believe that the phosphate retention is due to some disturbance in the specific function of the kidney and not to increased phosphate production in the body or increased absorption from the alimentary canal.

As will be seen from our analyses there is a tendency to higher than normal figures in patients suffering from nephritis and uræmia, and in post-choleraic uræmia the percentage of phosphate in the blood may be very considerably increased. The relationship of phosphate retention, however, to the clinical condition of the patient and to prognosis is too vague at the present time to enable us to arrive at any definite conclusion as to its bearing and importance. Many cases die where the retention is not very marked and seemingly may live when the phosphates of the blood and even the alkalinity are greatly disturbed. More extended investigations are necessary to clear up the significance and bearing of phosphate retention in nephritis and uræmia. The fact that we have obtained far higher figures for this retention in post-choleraic uræmia (which is largely suppression of excretion) than in the ordinary fatal forms of uræmia, and the further fact that many of these severe post-choleraic cases recovered, would point to the retention of phosphates as not being of outstanding importance as a factor in leading to a fatal issue. In all probability the retention of acid phosphates may account for the decrease in the chemical alkalinity of the blood, although even in this the figures so far obtained are not very convincing. Personally, we do not believe that the retention of phosphates is inherently connected with true acidosis, *i.e.*, the presence of acetone bodies in the blood.

The whole subject requires thorough investigation not as a laboratory exercise, but as one in which the blood analyses are carried out in the very closest relationship to the clinical signs and symptoms shown by the patient at the time the blood is taken for examination.

We may conclude from the analyses produced in Table V that in nephritis, whatever may be the cause, in uræmia of whatever origin, the blood picture is characterised by a retention of non-protein nitrogenous bodies of which urea may form a very great proportion, as in



suppression, cholera, etc., or of which the urea may form quite a small percentage, as in true parenchymatous nephritis.

We shall now turn to a consideration of the blood analyses in these cases where the patients were suffering from diabetes and where, under ordinary circumstances, they would have been classified as dying of diabetic coma, following on gangrene, etc.

In a previous communication, 'The Treatment of Diabetes in India,' published in this number, we have brought forward a large mass of evidence to show that the prevailing form of diabetes met with in India is of a very mild type; that patients rarely die from true diabetic coma: that acidosis to any serious degree is very exceptional—acetone and di-acetic acid elimination in the urine being practically negligible. Yet these patients die and large numbers of them die in coma; though, even when coma is present, the urinary examination may show practically no acetone or  $\beta$  oxybutyric acid, and only once or twice, amongst our cases, was di-acetic acid present. In order to solve the mystery of the cause of death in cases of this type, the condition of the blood was investigated.

TABLE VI.

*The blood in diabetics dying in coma and in those in a grave condition.*

*Case 148.* Strong, healthy man, accustomed to exercise, but very stout. There is a history of glycosuria for five years, probably present longer. Had had cholera, small pox, gonorrhoea: operated on for urethral stricture six years ago. He had a large carbuncle about a year ago on the back.

About fifteen days before admission he noticed his right big toe and adjoining parts swollen and painful. Despite local treatment the condition grew worse, and he came to hospital.

Admitted on 9th March, 1917: The septic gangrenous condition of the foot and leg was opened up at once.

The patient appeared very toxic, was dull and stupid, had a nasty earthy cachectic colour: there was slight delirium and restlessness.

He showed no signs of air hunger: smell of sepsis present.

The history showed that he had been excreting urine freely up to a few days before admission to hospital.

The urine on admission contained sugar 2.5%, albumen distinct reaction, no casts, acetone present, di-acetic acid *nil*.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Fat.	Alkalinity.	N.P.N T.N.
	%	%	%	%	%		
10-3-1917 ..	2.97	.24	.13	.56	.17	n 25	$\frac{1}{12}$
11-3-1917 ..	3.10	.56	.25	.54	.54	..	$\frac{1}{5.5}$

*Urine Examination.*

Date.	Sugar of urine.	Albumen.	Acetone.	Urea.
	%		%	%
10-3-1917 ..	2.5	++	.0174	1.3
11-3-1917 ..	<i>nil</i>	++	trace	.3

This man quickly passed into stupor and coma. He died on the evening of the 11th March, 1917. The excretion of urine fell to 240 c.c. on the day he died.

Now, so far as it is possible to say, this patient was very little if at all worse, so far as his kidney function was concerned than he had been for years, or than practically any ordinary glycosuric very quickly becomes. That is, he suffered from the so-called simple albuminuria that accompanies glycosuria and which is generally considered fairly harmless. Yet the blood pictures reveal the condition to be identical with that of severe kidney disease and that the cause of death was uræmia.

It may be concluded that the so-called simple albuminuria may become a very serious condition and may lead to the onset of fatal uræmia in much the same way as true interstitial nephritis may do so.

In addition to a holding-up of the non-protein nitrogen and urea, it is worth drawing attention to the fact that, although the sugar disappeared from the urine before death, the sugar content of the blood

was very high, 0.54 per cent. The high percentages of fat and waste-products were probably due to tissue breakdown.

*Case 149.*—The patient was a big fat man, seemingly healthy. Hindu, aged 42 years. Up to a year ago had been quite healthy and normal. Polyuria began a year ago, and he noticed his appetite increased, but he did not know there was glycosuria up to admission to hospital.

He gives a history of being subject to boils. A month before admission his scrotum began to be inflamed; the infection spread and became septic.

Admitted 10th May, 1917, in a serious condition. Septic condition of scrotum and adjoining parts. The patient was very toxic, the temperature only slightly raised. He was semi-conscious and tremulous.

The respirations varied from 32 to 40 during the few days he lived after admission, the pulse was 96 to 100, temperature 100 to 101°F.

There was no air hunger and no smell of acetone.

#### *Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Fat.	N.P.N. T.N.
	%	%	%	%	%	
12-5-1917 ..	2.28	177	.22	.63	.24	$\frac{1}{13}$
13-5-1917 ..	2.24	181	.25	.83	.20	$\frac{1}{12}$

#### *Urine Examination.*

Date.	Sugar of urine.	Albumen.	Acetone.	Urea.
	%	%		%
12-5-1917 ..	nil	.05	++	1.5
13-5-1917 ..	nil	.30	++	+

The patient gradually passed into coma from the dull sleepy state he was in on admission and died next day.

This patient showed more albuminuria than No. 148 but nothing pointing to real nephritis. The blood picture is again that of uræmia, and except for a trace of acetone in the urine he showed no signs of real acidosis. Clinically he had marked toxæmia and the coma is looked upon as diabetic in these cases, once glycosuria and ketonuria are discovered. The high hyperglycæmia without glycosuria is again very noteworthy.

*Case 150.*—Hindu male, aged 50. History of polyuria for five years. Admitted for gangrene of the penis. The patient's condition was very bad. He was toxæmic and cachetic. This man lived for over five weeks and at one time improved considerably, but eventually sank into coma and died.

Admitted 26th May, 1917, died 4th July, 1917.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Fat.	N.P.N. T.N.
	%	%	%	%	%	
26-5-1917 ..	3.05	.047	.042	.24	.16	$\frac{1}{65}$
5-6-1917 ..	2.24	.061	.012	.32	.08	$\frac{1}{37}$
16-6-1917 ..	2.46	.168	.027	.22	..	$\frac{1}{15}$

*Urine Examination.*

Date.	Sugar of urine.	Albumen.	Acetone.
	%		
26-5-1917 ..	nil	+ +	nil
5-6-1917 ..	.8%	+ +	nil
16-6-1917 ..	.4%	+ +	nil

The alkalinity of the blood and phosphates were normal. The patient lived for nearly three weeks after the last blood examination, but died in a similar manner to Nos. 148 and 149.

In this case the non-protein nitrogen of the blood was being retained out of all proportion to the urea nitrogen. Doubtless, later, the urea would also increase to a much higher percentage than when the blood was examined. We have noticed that the non-protein nitrogen is usually the first to be retained and later, as the excretion of urine fails, the urea accumulates.

This patient also died in coma— the blood picture being that of uræmia.

*Case 151.*—The patient was admitted for diabetic gangrene of the foot and leg. His condition was very bad, drowsy, stupid and semi-delirious. His friends took him home to die on the day he was admitted.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Phosphates.	Chlorides.	Alkalinity.	N.P.N. T.N.
		%	%	%	%	%	"	
21-6-1917	2.32	.14	.04	.56	.015	.625	.30	1 15

*Urea Examination.*

Date.	Sugar of urine.	Albumen.	Acetone.	Urea.
	%			%
21-6-1917	4.8	++	++	.95

This was evidently a similar type of case to those already given. The patient left hospital and probably died in a few days.

*Case 152.*—Very dark Eurasian: admitted for cellulitis of the shoulder which was not extensive. He was very toxæmic and uræmic in appearance. Low muttering delirium and typhoid state. He sank rapidly and died on day of admission. His breathing was very rapid which was due to an extensive pericardial exudate. The blood was obtained just before he died and pericardial fluid after death.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Fat.	N.P.N. T. N.
	%	%	%	%	%	
19-3-1917 ..	2.77	.46	.225	.28	.18	$\frac{1}{6}$

*Urine Examination.*

Date.	Sugar of urine.	Albumen.	Acetone.
19-3-1917 ..	Present.	Present.	Present.
Urea of pericardial fluid .. .18%			

The quantity of urine obtained was very small.

There was no smell of acetone in the breath and no di-acetic acid in the urine.

Here again a patient dies in coma, sugar and acetone are found in the urine: the obvious diagnosis is diabetic coma. The blood picture however is that of uræmia.

*Case 153.*—Eurasian female, aged 30, married. Admitted for continuous vomiting, severe stomatitis and semi-consciousness. The patient was collapsed.

She had had several abortions: one child living and healthy. Seven years ago had puerperal fever and during one pregnancy had an attack somewhat similar to the present one. Since then has suffered from severe headaches. She suffered from pyorrhœa alveolaris and has had all her teeth extracted.

She was admitted on the evening of 19th July, 1917, and had more of the appearance of true diabetic coma than any case so far encountered.

The urine (catheter specimen) on admission showed:

Albumen	..	..	..	+++
Sugar	..	..	..	++
Acetone	..	..	..	++
Di-acetic acid	..	..	..	+
Casts	.. a few hyaline and granular.			

*Coma as a Cause of Death in Diabetes.*

This was the first case of this type of diabetes in which a positive reaction for di-acetic acid was obtained in the urine. The reaction was not very marked.

The temperature was 104°F.; respiration varied from 36 to 44, and the pulse 120 to 140. There was no typical air hunger, and no deep sighing respiration.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Phosphates.	Chlorides.	Alkalinity.	Ammonia of Blood.	Acetone of Blood.	N.P.N. T.N.
	g.	g.	g.	g.	g.	g.	n	%	g.	
11-7-1917	2.94	.21	.162	.86	.015	.66	45	.0023	.009	1 14

*Urine :—*

Albumen	traces
Acetone	+++
Casts	nil
Di-acetic acid	++
Ammonia	.016%

She died the same evening in coma.

This is a most important case from our standpoint. Everything pointed to the condition being diabetic coma. The patient was young; a glycosuric; her urine showed acetone and di-acetic acid. She had hyperpnœa and gradually deepening coma. What else could be the condition, if not typical acidosis and diabetic coma?

Yet the analysis of the blood opens up a very different aspect and places the cause of death as far more likely to be uræmic than diabetic coma.

That there is a certain degree of acidosis present our analyses show: thus, the urine and blood both contain a small percentage of acetone and the alkalinity of the blood is slightly higher than normal. On the other hand, it is well to remember that the patient had been vomiting and had had little to eat for some days before coming to hospital: it is extremely probable that this in itself is quite sufficient to account for the degree of acidosis present and that the diabetic state had nothing to do with it. That the acidosis was comparatively mild is evidenced



by the small percentage of acetone in the blood, and by the fact that the percentage of ammonia in the urine was practically normal. It will be observed that there was no retention of phosphates in the blood.

Here again the blood picture is that of uræmia, although the ordinary urinary findings would point to diabetic coma as the cause of death.

Except for sordes and a certain degree of stomatitis—non-septic—this patient differed from those previously described in that no septic absorption was present. Yet the signs of uræmia are very much the same as in the septic cases.

*Case 154.*—European male, aged 50 years. Admitted for diabetic gangrene of foot. There was considerable atheroma of the arteries. The patient was seriously ill on admission but improved for a time; then the gangrene began to spread upwards and on the 29th June, 1917, the leg was amputated.

He died on the 22nd July, 1917, after being in coma for three days before death.

#### Blood Examination.

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Phosphates.	Alkalinity.	Fat.	N.P.N. T.N.
	%	%	%	%	%		%	
19-6-1917 ..	2.67	1.56	.07	.263	.02	n 32.5	.12	1 17
27-6-1917 ..	2.73	.07	.048	.22	..	..	..	1 39
29-6-1917 ..	3.22	.084	.05	.28	.02	n 37.5	..	1 39
30-6-1917 ..	3.15	.19	.146	.22	.04	n 50	..	1 16

#### Urine Examination.

Date.	Albumen.	Sugar.	Acetone.	Urea.
	%	%		%
19-6-1917 ..	++	.8	+++	2.2
27-6-1917 ..	++	.8	++	..
29-6-1917 ..	.75	2.3	++	2.5
30-6-1917 ..	.20	trace	++	1.5

Di-acetic acid was absent throughout and the urine showed casts.

From the 29th June 1917 the amount of urine excreted greatly diminished and it showed the presence of bile.

The blood serum was yellow and contained bile.

Here again the ordinary diagnosis would be diabetic coma following on an operation, yet the chemical analysis of the blood points to uræmia as the cause of death.

*Case 155.*—European male, aged 65 years. Admitted for extensive diabetic gangrene of the foot and leg. He was very toxæmic and uræmic. Passed into coma, and died suddenly after transfusion.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Hct.	N.P.N. T.N.
18-11-1917 ..	2.30	.11	.07	.35	.48	$\frac{1}{21}$
23-11-1917 ..	2.10	.132	.095	.43	.38	$\frac{1}{16}$

*Urine Examination.*

Date.	Sugar.	Albumen.	Urea.	Acetone.	Ammonia.
18-11-1917 ..	"	"	"	"	"
23-11-1917 ..	+	+	2.5	+	0.1

There was no di-acetic acid.

The blood picture again simulates that of uræmia.

*Case 156.* Eurasian male, aged 38 years. Admitted for hemiplegia; aphasia was semi-conscious. There was no hyperpnoea. The urine on admission showed the presence of albumen, sugar and acetone. There was no di-acetic acid. He remained in hospital about a month, regained consciousness and left much improved; the hemiplegia also slightly improved.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Phosphates.	Chlorides.	Alkalinity.	N.P.N. T.N.
22-5-1917	3.61	.20	.063	.30	..	..	..	1 18
26-6-1917	3.13	.075	.052	.31	.0175	.575	0 30	1 11

*Urine Examination.*

Albumen	..	..	..	++
Sugar	..	..	..	4.6%
Acetone	..	..	..	++
Di-acetic acid	..	..	..	nil

This man was in a serious uræmic condition when admitted, yet from the analyses of the urine, his diabetes was considered the important condition.

He did well under treatment and passed from the acute dangerous uræmic condition to that of chronic hemiplegia.

*Case 157.*—Hindu, male, old diabetic. Admitted for diabetes in December 1917. He was seriously ill but reacted to treatment and left hospital fairly well.

In addition to diabetes he had albuminuria and his condition was due much more to renal trouble than to the diabetes.

Despite a fairly high hyperglycæmia, as a rule he passed sugar in his urine only after meals. In March he returned to hospital in semi-coma, which gradually deepened, and he died after three days.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Fat.	Ammoniacs.	N.P.N. T.N.
	%	%	%	%	%	%	
26-12-1917 ..	2.04	.152	.038	.29	.04	..	1 13
21-3-1918 ..	2.814	.136	.065	.42	.12	.033	1 21
23-3-1918 ..	2.80	.142	.05	.40	..	..	1 20

*Urine Examination.*

Date.	Albumen.	Sugar.	Acetone.	Cast.
	%	%		
26-12-1917 ..	·10	·80	<i>nil</i>	<i>nil</i>
21-3-1918 ..	·05	1·5	+ +	+ +
23-3-1918 ..	·025	2·0	<i>nil</i>	+ +

Here again the coma that ends the scene in this type of diabetes is uræmic.

*Case 158.*—A somewhat similar case. Admitted delirious, sank into coma and died. Aged 60.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Fat.	Acetone.	N.P.N. T.N.
	%	%	%	%	%		
2-3-1918 ..	2·85	·09	·075	·19	·16	—	$\frac{1}{32}$

*Urine Examination.*

Albumen ..	..	..	..	·025%
Glucose ..	..	..	..	2·5%
Acetone ..	..	..	..	·01%
Di-acetic acid ..	..	..	..	<i>nil</i> .

Again, from the urine diabetic coma was the condition suspected, whereas the blood picture is much more that of uræmia. He was taken from hospital in an unconscious condition and died four days later.

*Case 159.*—A young Bengali medical officer, aged 32 years. The family history of diabetes is very strong. Had eight brothers living who are all very fat and all have diabetes. The patient has had glycosuria for four years.

He was admitted for cellulitis of the neck following on boils, but only lived one day.

He was conscious up to the end, but very restless. The breathing was very hurried.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Acetone.	N.P.N. T.N.
	%	%	%	%		
5-3-1918 ..	3.15	.14	.035	.33	—	$\frac{1}{22}$

*Urine Examination.*

Date.	Albumen.	Sugar.	Acetone.	Di-acetic acid.
	%	%	%	
5-3-1918 ..	.025	.40	.06	+ trace

This patient had no coma : the blood picture however pointed to considerable retention of nitrogenous waste-products.

He died so suddenly that very little was known regarding the real cause of death.

*Case 160.*—Jew, 65 years of age. Admitted for diabetic gangrene of the foot, in a very low condition. He was semi-comatose ; low muttering delirium ; coma deepened and he died in two days.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Amino-acids.	Fat.	N.P.N. T.N.
	%	%	%	%	%	%	
28-3-1918 ..	2.80	.084	.065	.50	.013	..	$\frac{1}{33}$
29-3-1918 ..	2.66	.098	.09	.85	..	.32	$\frac{1}{27}$

*Urine Examination.*

Albumen ..	..	..	..	.15 <sup>0</sup> <sub>00</sub>
Glucose ..	..	..	..	4 <sup>0</sup> <sub>00</sub>
Acetone ..	..	..	..	.0012 <sup>0</sup> <sub>00</sub>
Di-acetic acid ..	..	..	..	nil

This case is very similar to the other patients noted on with gangrene. The free excretion of urine kept down the urea content of the blood to some extent.

The blood picture, however, is that of uræmia.

These thirteen cases, Nos. 148 to 160, are sufficient to illustrate our views regarding coma as a cause of death in the ordinary type of diabetes met with in this country. It has hitherto been taken for granted that a patient with stupor or coma, whose urine contains sugar or acetone or both, is suffering from diabetic coma. Not uncommonly this view is confirmed by a positive ferric chloride reaction for the presence of di-acetic acid—the real explanation of the positive diacetic reaction is, in 99·9 per cent of the cases, that the urine is alkaline when the ferric chloride is added. Alkaline urine gives a reddish colour with ferric chloride and thus the patient is said to be passing di-acetic acid when such is not the case.

We have given the results of the urine and blood analyses, and whilst our deductions may be wrong and some totally different interpretation be the correct one, we have no hesitation in stating that in our opinion the acidosis and diabetic coma, which are described as a common terminal phenomena of the diabetes of temperate climes, do not occur, or only very rarely occur, in India. The evidence for this view is overwhelming and may be found in this paper and in the article, already referred to, dealing with the treatment of the Indian type of the disease. We have failed to find any real signs of acidosis to a degree worthy of consideration, either in the ordinary diabetic under treatment or in those patients suffering from coma. We have gone further and have shown from the analyses of the blood that the coma is not due to acidosis but that it is uræmic.

This concludes our work on the blood conditions as met with under varying conditions—health, albuminuria, glycosuria, with or without an accompanying albuminuria, frank kidney disease with and without uræmia, and lastly the blood in the terminal stages of diabetes. The conclusion at which we have arrived is that there is practically no essential difference between the chemical condition of the blood in those dying from uræmic coma and those diabetics who die in coma. That is, that in the Indian or very mild type of diabetes those patients who develop coma and die are suffering from uræmia and not from a gradually increasing acidosis leading to diabetic coma.

Before concluding an account of our investigations on the condition of the blood we desire to give a short account of two cases of what appeared to be real diabetic coma that came under our observation. The contrast, in clinical appearances, between these and the so-called diabetic coma of India could not well be exaggerated. The real coma of diabetes, as exemplified in the two patients referred to, presented the usual classical signs of diabetic coma: marked acidosis, acetone smell of the breath, and even of the bed-room, air-hunger, restlessness, gradually deepening coma, etc.

Both these cases were Europeans and the only other cases of a similar nature seen—two in number—were also Europeans. One occurred in acute diabetes of only a month's standing; the other cases were examples of *diabetes gravis* of one to two years' standing. Unfortunately we were only able to obtain the blood in two of the cases and in one only whilst in coma. Before giving the results of our findings in these cases of true diabetic coma we may be permitted to draw attention to some points noted, but whose significance appears to us to have been missed, by Epstein and Baehr in their "*Studies in Experimental Diabetes after Pancreatectomy*," *The Journal of Biological Chemistry*, Vol. XXIV, 1916.

The authors, from the results of some very successful experiments in total pancreatectomy, show that the volume of the blood increases very markedly so that allowances must be made in order to obtain an accurate idea of the increase in its several constituents; also they find that the hyperglycæmia mounts progressively and is followed by a marked terminal rise, due to a *diminution of the permeability of the kidneys*. (Italics are ours.)

What we are concerned with, however, is not the mobilization of sugar, the hyperglycæmia and its terminal rise, but with the effects of pancreatectomy on the kidneys and their function of purifying the blood. Thus complete extirpation of the pancreas was performed on a cat, which was allowed to live for 141 hours. The following results were obtained:

- (i) The blood sugar rose from '084 per cent to '838 per cent.
- (ii) The volume of the blood increased 150 per cent.
- (iii) *The non-protein nitrogen of the blood increased to '324 per cent.*
- (iv) The excretion of urine after four days decreased from 10 c.c. per hour to 1'34 c.c. per hour; at the same time, the



excretion of sugar and acetone fell to *nil* and the albuminuria, absent for the first 48 hours, gradually got worse until marked albuminuria was present. Casts also appeared.

(v) Stupor set in during the last 12 hours of the experiment.

These are most interesting findings in the light of our blood analyses in the terminal stages of diabetes.

Here is a healthy cat which shows no signs of kidney trouble for 48 hours after pancreatectomy; then albumen and casts make their appearance in increasing amounts. As further evidence of progressive derangement of kidney function, the excretion of sugar and acetone falls gradually to traces and then *nil*. The degree of albuminuria increases; practically anuria supervenes, and the animal passes into stupor. Soon afterwards the cat is killed and the non-protein nitrogen of the blood is found to be 32.1 per cent—a probable relationship  $\frac{\text{N.P.N.}}{\text{T.N.}} = \frac{1}{5.8}$ . This simulates very closely the condition we found in uræmic coma and in the so-called diabetic coma of India.

This confirmation of our findings should assist in focussing attention on the importance of the so-called simple albuminuria of diabetes, which is said to be of no great significance and to be no evidence of serious derangement of the renal functions.

We hold the very opposite opinion and regard albuminuria simple or severe, as of very great importance. In India, the presence of albumen is of far greater prognostic significance than the presence or the percentage of sugar; and, when there is any tendency to stupor or coma, albuminuria, with or without casts, is a very serious complication and the coma supervening thereon is very unlikely to react to treatment, unless the excretory functions of the kidney can be restored.

Again, we say that, in the very purest forms of true diabetic coma, in our opinion, there is a very large uræmic factor; and, in the type of diabetes prevalent in India, the uræmic element entirely overshadows the acidosis of real diabetic coma.

*Case 161.*—Diabetes, exhibiting the usual signs of acidosis.

European, male, aged 54 years. Admitted for severe diabetes. The patient, so far as he knew, had been healthy up to 1914. He blames the drinking of sour wines while travelling on the continent for being the cause of his trouble. His stomach and digestion were badly upset

on arrival in India : he began to lose weight and very shortly presented the usual signs : polyuria, polyphagia, etc.

Sugar was discovered as soon as he saw a medical officer, and he was treated up to January, 1918, privately. When first seen by us the patient was very seriously ill ; he was much emaciated, was very weak, and he had a well-marked bronzed appearance. He was passing large quantities of urine which contained over 7 per cent of sugar ; acetone and di-acetic acid were also present.

The blood and urine were first examined by us on the 12th January, 1918.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Acetone.	Fat.	N.P.N. T. N.
	%	%	%	%		%	
12-1-1918 ..	2.57	.14	.046	.39	+++	.22	$\frac{1}{17}$

*Urine Examination.*

Date.	Albumen.	Sugar.	Acetone.	Urea.	Di-acetic acid.
		%		%	
12-1-1918 ..	+	7.2	+	.8	+

He was admitted into Hospital on 23rd January, 1918.

*Blood Examination.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Urea.	Glucose.	Acetone.	Fat.	N. P. N. T. N.
	%	%	%	%		%	
26-1-1918 ..	2.20	.154	.04	.156	+	..	$\frac{1}{14}$
11-2-1918 ..	3.15	.175	.04	.24	+	.20	$\frac{1}{18}$
24-3-1918 ..	3.06	.152	.04	.22	..	.12	$\frac{1}{20}$
31-3-1918 ..	3.08	.144	.03	..	..	..	$\frac{1}{21}$

Amino-acids of blood, .008 per cent.

*Urine Examination.*


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Date.	Albumen.	Sugar.	Acetone.	Di-acetic acid.
<hr/>				
26-1-1918	..	+	+	.6
11-2-1918	..	..	1	+
24-3-1918	..	trace	1.6	nil
31-3-1918	..	trace	nd.	+

---

On the whole the patient did fairly well under treatment. The glycosuria disappeared entirely for a time, the acetone fell to only a trace, and the di-acetic acid disappeared except for occasional traces. The patient's general health improved and he gained strength. He never looked healthy and always seemed to be living on the brink of disaster. It was practically impossible to re-educate his carbohydrate tolerance above 60 to 65 grammes per day. He left hospital on the 9th April and went to the hills for seven months. The patient lived on the diet prescribed, except for occasional lapses, and was able to enjoy life in a mild way. Occasionally sugar recurred and there were often traces of acetone and di-acetic acid.

In November he returned to Calcutta looking very frail and emaciated but on the whole feeling better. He went back to his work for a couple of months and also unfortunately to worry. He relaxed his dietetic restrictions and in January 1919 suddenly began to get dull and sleepy. This rapidly got worse and he died a few hours after admission into hospital in typical diabetic coma. The urine contained large quantities of acetone and di-acetic acid, and his breath smelled strongly of acetone.

Unfortunately no blood examination could be made as he was admitted into another hospital where there were no facilities for carrying out the necessary analyses.

Such is the history of this case which was under our observation for a year. During the last two months little was known of the patient otherwise he might have been prevented from risking the development of coma. So long as he adhered to the line of treatment which was found to suit his condition the patient, although thin and emaciated, kept wonderfully well, fairly strong and very cheery. He was quite able to walk

a couple of miles at a time whilst in the hills, and he was in much better health and physical condition than when first admitted into hospital.

Although we had not the opportunity of seeing the patient after he developed coma, from the account of his state there is little doubt but that he had real diabetic coma with the usual marked signs of acidosis.

Yet from what we knew of the chemical condition of his blood whilst under our care, it would appear very probable that the retention of nitrogenous waste-products had a considerable influence in determining the onset of coma, and in leading to a fatal issue.

*Case 162.*—Acute diabetes gravis presenting all signs of diabetic coma.

Just as the writing of this paper was nearing its conclusion a case of acute diabetes gravis was transferred at our request from another hospital to the Medical College Hospital.

The history is a very short one: the man—again a European—was all right up to the 12th January, 1919. He then began to pass large quantities of urine and always felt hungry. This continued for some weeks, but, although feeling tired and without energy, he stuck to his work until his superior officer ordered him to see a physician. Sugar was discovered and he was admitted into a local hospital for treatment. Despite rigorous dietetic restrictions, starvation, etc., the patient rapidly grew worse: the glycosuria was uncontrollable and he quickly became emaciated. He continued to pass large quantities of urine with a high percentage of sugar—3 to 4 litres per day with 6 to 8 per cent of sugar—in spite of the most careful dieting. Acetone and di-acetic acid were present in the urine.

He was transferred to our charge on the 21st February, 1919. On admission he looked very weak, anæmic and emaciated. His colour was clear and pale, eyes bright and shining. There was a distinct acetone smell from his breath.

The patient, aged 24 years, gave no history of illness immediately preceding the onset of the disease. For the five days previous to admission to this hospital he passed an average of 120 oz. of urine per day, in which the sugar increased from 31 to 44 grains to the ounce: this was despite rigorous dieting.

He weighed 98 lb. on admission, a loss of over 30 lb. since his illness began on the 12th January, 1919.

On the 22nd and 23rd February the patient was very restless, complained of distension, flatulency and pain in the back. A flatus tube, enemata and stupes were ordered. The bowels were moved.

On the 24th he complained of great pain and tenderness on the right side: this was found to be due to early pleurisy and pneumonia—the next day he was coughing up blood.

On the morning of the 25th February the patient was very dull, listless and sinking into stupor. The breathing became deep and sighing and he rapidly passed into coma. The breath smelt strongly of acetone and, as will be seen from the record of urine analyses, large quantities of acetone and di-acetic acid were being excreted by the kidneys.

*The daily analyses of the urine.*

Date.	Quantity, c. c.	Sugar.	Quantity, grm.	Acetone.	Quantity, grm.	Ammonia.	Quantity, grm.	Di-acetic acid.	Urea.	Quantity, grm.
		%		%		%			%	
23-2-1919..	8200	7.3	598.6	+++	+++	.098	8.03	++++	1.2	98.4
24-2-1919..	4400	7.0	308.0	.168	8.39	..	..	++++	..	..
25-2-1919..	4600	6.6	293.6	.201	9.24	.42	18.48	++++	1.2	56.2
26-2-1919..	8200	2.5	205.0	.148	12.13	..	..	+++	..	..
27-2-1919..	6200	2.5	155.0	.0406	2.51	.098	5.97	+++	1.0	62
28-2-1919..	5300	1.2	63.6	.077	1.08	.095	5.02	+++	..	..
1-3-1919..	3900	1.8	70.2	.1014	1.07	.1292	5.03	+++	..	..
2-3-1919..	3700	1.2	44.4	.085	3.14	.136	5.03	+++	1.2	44.4
3-3-1919..	5000	1.0	50.0	.058	2.90	alk.	..	++	.8	40.0
4-3-1919..	6200	.8	49.6	.052	3.20	.034	2.10	+	.6	37.2
5-3-1919..	4700	.3	14.1	.0328	1.64	alk.	..	+	.65	30.5
6-3-1919..	4000	.2	8.0	.0077	0.30	.023	.92	nil.	1.2	18.0
7-3-1919..	2700	.3	8.1	.0096	0.25	alk.	..	nil.	1.0	27.0
8-3-1919..	3200	.3	9.6	.0053	0.16	alk.	..	nil.	1.0	32.0
9-3-1919..	2400	trace	trace	trace	trace	alk.	..	nil.	.6	14.4
10-3-1919..	3200	nil.	nil.	nil.	nil.	alk.	..	nil.	.6	19.2
11-3-1919..	4200	nil.	nil.	nil.	nil.	alk.	..	nil.	.6	25.2
12-3-1919..	2650	nil.	nil.	nil.	nil.	alk.	..	nil.	.7	18.5

*The chemical condition of the blood.*

Date.	Total Nitrogen.	Non-protein Nitrogen.	Glucose.	Urea.	Acetone.	Phosphates.	Alkalinity.	CO <sub>2</sub> Capacity.	N.P.N. T.N.
	%	%	%	%					
23-2-1919 ..	2.94	.098	.56	.04	+++	.015	n 37.5	27	$\frac{1}{30}$
25-2-1919 ..	2.90	.112	.26	.039	+++	.015	n 35	35	$\frac{1}{26}$
27-2-1919 ..	2.96	.116	..	..	++	.015	n 35	..	$\frac{1}{26}$
4-3-1919 ..	2.56	.110	.22	.04	..	.015	n 25	80	$\frac{1}{23}$

No time was lost, an intravenous injection of a pint and a half of a 3 per cent sodium carbonate solution was given and the alkali treatment increased. The effect was fairly well marked; the patient gradually recovered from the coma. The alkali therapy was continued—sodium bicarbonate in 25-grain doses were given every three hours until the urine became alkaline.

On the 26th and 27th February, the condition had improved but on the 28th vomiting set in and later again stupor and coma began to supervene. Another intravenous of a pint of the same solution was given. Distinct improvement was noted on the following two days, the patient became conscious, coherent and the vomiting ceased. Of course, the pneumonia did not help to make matters any simpler, but, on the whole, he was distinctly better than on admission although greatly wasted and emaciated.

On the 4th March the patient was considerably better. The urine examination shows a marked decrease in acetone and di-acetic acid, and for the first time since the day before coma first supervened, there was an absence of albuminuria. The condition of the lung also showed considerable improvement and the cough, which was very troublesome, had almost disappeared.

On the 5th, 6th and 7th March improvement was maintained, although there was a considerable extension of the pneumonic patch. Glycosuria had practically disappeared, there was no di-acetic acid and only a trace of acetone in the urine.

Up to the 7th March the patient had almost no carbohydrates: a few ounces of milk, a little bovril and alcohol was all he could take. On the other hand he drank large quantities of water, soda water and weak tea.

We now tried to increase his food and gave him milk 15 oz., green vegetables 10 oz., alcohol 3 oz., a few spoonfuls of bovril and one egg.

When admitted the condition of mouth, tongue and lips was very bad; despite energetic treatment septic parotitis developed and a small operation was necessary on the 8th March. No anæsthetic was administered.

By the 10th of March all signs of acidosis had disappeared and the urine was free from sugar. Although still very weak and emaciated he improved daily and gained strength.

Such is the history of a most remarkable case of acute diabetes in its most virulent form.

What the cause may have been for such an acute onset and for the severity of the attack it is impossible to say. It is probable there was complete failure of the pancreatic internal secretion: the pituitary also may have been involved.

We have only given an outline of the case as it developed. We hope later to place on record all the points connected with its further history and the effects of treatment in restoring his carbohydrate tolerance.

The salient features are:—

1. Acute diabetes gravis of a very aggravated type.
2. Complete loss of carbohydrate tolerance.
3. Body-tissues rapidly broken down and the sugar eliminated in the urine up to at least one hundred times the quantity taken in by the mouth.
4. Very great polyuria—out of all proportion to the hyperglycæmia. This is important from the standpoint of the original cause, *i.e.*, whether other organs of internal secretion, such as pituitary, were not involved in addition to the pancreas.

It is also important from another standpoint, *viz.*, the recovery of the patient from two attacks of coma. If the excretion of the urine had failed, even though it was a very watery excretion, containing only a low percentage of nitrogenous waste products, it is very unlikely that the patient would have survived.

5. The daily records of the urine examination disclose a remarkable and, as far as our experience goes, a unique state of affairs.



The patient was, to all intents and purposes, starved from the 21st February to the 6th March. He occasionally retained a little milk; but except for a few ounces of alcohol and plenty of fluids he vomited most of his food for some days. Yet, during that time he lost over 1,850 grammes of sugar and the quantity of urea passed shows how rapidly his tissues were breaking down. The decrease and final disappearance of the sugar from the urine is also most remarkable in a case of such severity.

Similarly with regard to the acetone and di-acetic acid. Never before has anything approaching the findings above recorded been encountered by us. They show a very severe acidosis and this is borne out by the great increase in the ammonia present in the urine. No such conditions are ever met with in the ordinary mild type of diabetes prevalent amongst Indians.

6. The blood examinations show that although there was strong evidence that acidosis was the cause of the coma, yet the question of uræmia cannot be passed over in even the most typical forms of diabetic coma. The analyses disclose the fact that, despite the severe polyuria, there was a great accumulation of non-protein nitrogenous waste-products in the blood. The fact that the coma ended satisfactorily and was amenable to treatment was, in our opinion, due to the activity of the kidneys in eliminating acetone, di-acetic acid and nitrogenous waste-products. If the excretory powers of the kidneys had failed and the non-protein nitrogen retention had increased, all the evidence afforded by our analyses shows that death would have been inevitable.

7. The lesson we learn from this case with regard to diabetic coma is that, in all probability, coma, its onset, duration and termination, is dependent on the eliminative powers of the kidney.

Probably even the most typical forms of diabetic coma are largely uræmic in nature: the acidosis part of the coma is the part amenable to treatment; so that, as in this case, once the acidosis was brought under control—the uræmic conditions not being sufficiently severe—treatment was followed by most satisfactory results.

We suggest therefore that the typical diabetic coma of diabetes gravis is uræmic in nature but, in contradistinction to the coma of the mild Indian form of diabetes which is entirely uræmic, that there is an accompanying acidosis.

What part the acidosis plays outside its influence on the so-called 'buffer-salts,' and the elimination of  $\text{CO}_2$  from the blood and tissues, it

is difficult to say. The acidosis probably does account for the hyperpnœa, air-hunger, restlessness, and perhaps the rapidity of onset, but, we believe, the uræmic element has much to say to the fatal termination and to the inefficacy of the ordinary methods of alkaline therapy.

#### METHODS OF WORK.

Five to twelve c.c. of blood were drawn from a vein, according to the particular analysis to be carried out.

Total nitrogen was estimated by the Kjeldahl process.

Non-protein nitrogen, after complete precipitation of protein by alcohol (absolute), then by saturated solution of chloride of zinc and absolute alcohol. The filtrate is estimated by Kjeldahl.

Urea. By the urease method.

Sugar. By the process already described. Phosphates and chlorides by a micro-method described by Captain J. A. Shorten. I.M.S. (*Indian Journal of Medical Research*, 1918).

Alkalinity. Wright's method.

Fat was estimated from the precipitate obtained during the sugar determination, by extraction with ether.

#### SUMMARY AND CONCLUSIONS.

1. The absence of any well-marked signs of acidosis either during the course and treatment of diabetes, or even in the terminal coma, raised our suspicions that the coma, as a terminal phenomenon in the type of diabetes prevalent in India, was not identical with diabetic coma as seen in Europe. Other considerations pointed to the probability of the coma being uræmic.

2. In order to obtain positive information a detailed chemical analysis of the blood was carried out; and, to secure the necessary data for the formation of a definite opinion, a very large number—over four hundred—blood analyses were made, covering the blood in health, simple glycosuria, glycosuria with slight albuminuria, slight albuminuria without glycosuria, severe kidney disease and uræmia, and glycosuria with coma or in those very seriously ill. The latter class, one and all, showed more or less albuminuria.

3. The estimation of the percentage of the urea content of the blood was found to be insufficient for the reasons mentioned in the early part of the text.

4. The retention of uric acid and creatinin in uræmia is discussed.

5. The retention of non-protein nitrogenous waste-products is discussed and the relationship of non-protein nitrogen to total nitrogen— $\frac{\text{N.P.N.}}{\text{T.N.}}$ —is brought forward as a standard. This gets over the difficulty of determining the degree of dilution of the blood that occurs when retention of salts or waste-products takes place.

6. We take early exception to the statement "simple diabetic albuminuria is a benign process often transient and stands in no known relation with true nephritis."

Our work shows that, probably without any true nephritis, the simple albuminuria may be a sign of a functional derangement of the kidney, which is sufficiently malignant to lead to all the usual signs of retention of nitrogenous waste-products and eventually to fatal uræmia.

7. Table I gives the blood analyses of twenty-five practically healthy individuals and the figures obtained may be accepted as normal standards for the different chemical constituents of the blood. Those we are particularly concerned with are the total nitrogen, non-protein nitrogen, urea, glucose, phosphates, chlorides, alkalinity, fat and the relationship  $\frac{\text{N.P.N.}}{\text{T.N.}}$ . The percentages of these different constituents are very similar to those obtained by other observers: the relationship  $\frac{\text{N.P.N.}}{\text{T.N.}} = \frac{1}{100}$  to  $\frac{1}{150}$  in health. This we regard as an important standard for comparison with the ratio that obtains when albuminuria is present.

8. Table II presents the blood analyses of ten cases of simple glycosuria in which the urine did not give a reaction for albumen. These cases rarely come to hospital: only when albuminuria is present does the glycosuric in India begin to think it time to seek advice and treatment.

On the whole the figures for non-protein nitrogen, urea, and the ratio  $\frac{\text{N.P.N.}}{\text{T.N.}}$  show a tendency to exceed the normal: this is probably not due to the glycosuria *per se*, but to the effect that glycosuria has on causing derangement of the excretory function of the kidneys, and thus preventing the proper elimination of nitrogenous waste-products. That is, before the advent, or after the appearance, of the gross sign of kidney derangement—albuminuria—changes are at work which tend to cause a retention of non-protein nitrogenous bodies; under perfectly healthy conditions these would have been eliminated.

9. In Tables III and IV we take up the chemical condition of the blood in those cases where this gross sign of deranged kidney function—albuminuria—is present.

Table III presents those where glycosuria was absent, and Table IV those where, in addition, there was diabetes.

The facts brought forward in these two tables may be considered together, as they run fairly parallel.

Whether there is glycosuria or not, once albuminuria is present, showing renal derangement, there is always a greater or less tendency—depending doubtless on the degree of renal involvement—for the retention of non-protein nitrogen, or urea, or both to occur with the result that the ratio  $\frac{\text{N.P.N.}}{\text{T.N.}}$  rises to quite a high figure.

We did not find much evidence of an increase in the phosphatic content of the blood or any marked tendency to a decrease in alkalinity, as measured by Wright's method, amongst the 96 blood analyses carried out in these cases.

On the other hand, we did find that anti-diabetic treatment—milk and green vegetables had a very favourable influence in lessening the retention of nitrogenous waste-products and in assisting in their elimination.

In some cases the non-protein nitrogen retention was very marked without the retention of urea being greatly increased: in other cases both were retained.

10. Table V deals with the chemical conditions of the blood in uræmia and in cases of severe kidney involvement.

As will be seen, there is a still greater tendency to a retention of non-protein nitrogen with or without a considerable increase in the urea of the blood.

The ratio  $\frac{\text{N.P.N.}}{\text{T.N.}}$  may reach a very high level in the severe cases of uræmia,  $\frac{1}{5}$  to  $\frac{1}{4}$ , being not uncommon.

Also, in severe cases, there is a retention of phosphates and often a decreased alkalinity is present. In fact all constituents are more or less increased, except the percentage of total nitrogen.

In uræmia of cholera, anuria from whatever cause, eclampsia, the retention of non-protein nitrogen, urea and phosphates and the decrease in alkalinity are usually well marked.

In some cases we found acetone in the blood and urine, in others—just as seriously ill—no acetone was present.

The presence of acetone is probably accidental, depending on starvation, vomiting, etc., and is no integral part of the clinical picture of uræmia.

The one constant outstanding feature of all cases of uræmia was a great increase in the ratio of the non-protein nitrogen to the total nitrogen of the blood. All other abnormalities varied from case to case: urea may be greatly increased or only seemingly slightly; phosphates and alkalinity may be distinctly changed—usually an increase in the percentage of phosphates is accompanied by a decrease in the alkalinity of the blood; signs of acidosis may be present or absent; the non-protein nitrogen, on the other hand, always shows an increase and, in severe cases, a great increase.

11. In Table VI we present the salient clinical features, and the chemical condition of the blood and urine in thirteen cases (Nos. 148 to 160) of the terminal stages of diabetes as usually seen in India.

From the findings set forth, coupled with the absence of any real signs of acidosis in Indian diabetics (*vide* 'The Treatment of Diabetes in India' in this number), we have arrived at the conclusion that acidosis and diabetic coma practically do not occur in India; but that, on the other hand, the coma that commonly ends the scene in India is uræmic and not diabetic.

We can find no essential difference between the chemical condition of the blood in those with uræmia and in those dying in coma who have been suffering from diabetes, except for the presence of hyperglycæmia in the latter, and this can hardly be regarded as a cause of coma. Again, the outstanding feature of these dying diabetics is the great increase in the non-protein nitrogen of the blood. Acetone, urea, phosphates, etc., may be increased to a greater or less extent, but the signs of acidosis are far too slight to enable one to ascribe the coma to any ill-effects caused by a meagre retention of acetone bodies in the blood.

We, therefore, arrive at the conclusion that the functional derangement of the kidney that accompanies diabetes is the all-important factor in producing the necessary conditions of the blood that lead to coma and death.

The type of coma most commonly met with is that characterised by restlessness followed by drowsiness and gradually deepening coma. The respirations may be quickened slightly: there is practically never any typical 'air hunger.' Cheyne-Stokes respiration may be present in some cases. The patients become incoherent and slightly delirious before sinking into coma.

12. We give notes and the analyses of the blood and urine of two patients suffering from diabetes gravis, both of whom developed a

condition corresponding with the true type of diabetic coma, including typical Kussmaul's 'air hunger.'

From the chemical condition of the blood and the presence of albuminuria it would appear that, in all probability, there is a very considerable uræmic element in even the purest form of so-called diabetic coma.

The second case, No. 162, is a most remarkable one and of outstanding interest and importance.

This patient developed suddenly an attack of acute diabetes gravis. In a few weeks he lost about one-fourth of his body-weight and from his arrival in hospital until the urine was free of sugar—18 days—he passed roughly 3,600 grammes, or about eight pounds of sugar.

Clinically, he had on the 25th February, 1919, seemingly a typical attack of diabetic coma, with all the usual signs one associates with that condition. The smell of his breath, the analyses of the urine, the air hunger, gradually deepening stupor, etc., all pointed to severe acidosis, which, in reality, was precipitated suddenly by an attack of pneumonia.

Recovery from the first attack was incomplete, when on the 28th stupor and coma again supervened. From this condition he recovered completely and by the 6th March the patient was in a very different condition. The glycosuria had almost disappeared; di-acetic acid had gone completely; acetone in traces alone remained, and the clinical signs of acidosis were entirely absent.

The chemical condition of the blood, even in this seemingly typical case of diabetic coma, shows exactly the same tendency to retention of nitrogenous waste-products as was found to be the case in those dying in coma, whether uræmic or the so-called diabetic coma of the Indian form of the disease.

The only difference we can see is that, in the coma of the Indian type of diabetes, signs of acidosis are either entirely wanting or only very slight in degree, whereas, in the typical diabetic coma of diabetes gravis the signs of acidosis are well marked. In both there is very great retention of nitrogenous waste-products.

We have not had a sufficient number of cases of the typical form of diabetic coma in which the condition of the blood could be investigated to enable us to speak positively, but, from the evidence we have been able to bring forward, it would appear that—

- (i) In the mild Indian form of diabetes a terminal coma is almost invariably uræmia.



- (ii) In the more severe forms of diabetes the coma is uræmic, complicated by a varying degree of acidosis.
- (iii) In diabetes gravis the coma is also uræmic in nature but, in addition, there is associated with the uræmia marked signs of acidosis. How far the acidosis acts as a factor in either causing the coma or in leading to a fatal issue is an open question. Personally we believe the uræmic moiety of the condition is sufficient to explain the coma and be the cause of death, and that the accumulation of acetone bodies in the blood is probably responsible for a more sudden onset of coma than would otherwise occur, also that it may account for the decrease of the 'buffer salts' and thus lead to hyperpnoea and Kussmaul's 'air-hunger.'

That the presence of even a large amount of these acetone bodies in the blood, or that a depletion of the alkali bases of the body caused by the struggle to eliminate the foreign acetone bodies—that either of these conditions is sufficient to account for typical diabetic coma and death, we are very disinclined to believe.

It appears to us that the influence of the renal elimination has not been taken sufficiently into account in any of the various forms of coma that may terminate diabetes, and that it will be essential in future to pay more attention to the retention and accumulation of nitrogenous waste-products within the body than to the possible depletion of the body of so-called 'buffer salts' which is supposed to lead to acidosis and coma.

We would suggest that the favourable influence exerted by the starvation of patients on the verge, or in the early stages, of diabetic coma, so long as the kidneys continue to act, can best be explained on our hypothesis, *viz.*, that the coma is uræmic in nature. The injection of alkaline solutions, normal saline, etc., also, so long as they cause a free flow of urine, will assist in eliminating the poisonous nitrogenous bodies and thus their beneficial effects in treatment are made evident: so far, of course, as they neutralize any acid bodies present in the body they will also assist.

On the other hand, if the excretory functions of the kidney have become seriously disturbed, the injections of these solutions is useless and no line of treatment is of any avail.

We have been careful in all we have said above to abstain from predicated that any serious pathological condition of the kidneys is the



forerunner of the onset of coma. Such, we believe, is by no means essential. Many of our cases died in what appeared to be typical uræmia, yet clinically the condition of the kidneys, so far as could be judged from the urine, œdema, heart and circulation, etc., was far from desperate.

In this very patient, No. 162, albuminuria only appeared two days before the onset of coma, casts were never found, and the albumen disappeared from the urine a few days after the second attack of coma, as soon as the clinical condition of the patient improved.

It is for this reason that we state that frank nephritis is not essential for the precipitation of an attack of uræmia, but, that the functional changes that accompany or permit of transient albuminuria are quite sufficient to prevent the elimination of the nitrogenous toxic bodies that, in all probability, are the cause or, at least, are the ever-present companion of uræmia.

# THE TREATMENT OF DIABETES IN INDIA.

BY

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On the 1st January, 1915, under the direction of the Scientific Advisory Board and at the expense of the Indian Research Fund Association, we began an enquiry into the causes of the exceeding prevalence of diabetes in India. Owing to the great pressure of work whilst the war lasted, it was found impossible to afford the time necessary to prepare our observations and results for publication. Since the pressure relaxed we have sent two papers to press in which we deal with the work carried

out with a view to solve the problem of the cause of the prevalence of diabetes in this country.

In a previous paper, published in Vol. VI, No. 4, of *The Indian Journal of Medical Research*, we arrived at the general conclusion that a prolonged and excessive carbohydrate dietary, combined with want of exercise, a sedentary life, and hereditary predisposition, is the main factor in the causation of the disease. The incidence of diabetes amongst the indolent, well-fed classes; its comparative rarity amongst the working classes; the very fact that the meat-eating Mahomedan, living under otherwise identical conditions with the Hindu, as regards climate, liability to malaria or other diseases, mental shocks, etc., escapes whilst his indolent, sugar-sodden companion develops the disease; all these point with increasing strength to the causative influences exerted by the ill-effects of a prolonged and excessive, one-sided, carbohydrate dietary. Whether, as we are inclined to believe, diet plus an indolent life is sufficient to throw such a great strain on the internal secretion of the pancreas as to weaken the pancreatic activity, decrease the output of the necessary amboceptors, and thus lessen carbohydrate oxidation, or whether some other factor of a more acute pathological nature is required, we are not prepared to say.

It is worthy of note, however, in this connection how very often in our experience there was apparently a close association between the one-sided carbohydrate type of diet and decreased hydrochloric acid of the gastric juice, fermentative dyspepsia, oxaluria or renal calculus, hyperphosphaturia, and glycosuria. Reference to the paper mentioned above will show that we have brought forward evidence to show how close this association often is; and we have offered as an explanation the possibility of a functional or organic disturbance of the pancreatic secretion, due to atrophic changes following on an extension of the chronic inflammation of the gastro-intestinal tract from the duodenum up the pancreatic duct.

Whatever may be the actual exciting factor, it is remarkable how very commonly patients with diabetes give a history of dyspepsia or some form of stomach troubles preceding the onset of glycosuria.

The commonly met with oxaluria, or alternating oxaluria and phosphaturia, we have shown, is simply a sign of the gastro-intestinal catarrh that so often precedes glycosuria. The evidence on the whole would point to some form of slow atrophy of the pancreas rather than an acute infection: there is seldom fever, the activity of the internal

secretion of the pancreas decreases gradually up to a certain point, and does not come on suddenly and, after a period of severe pancreatic depression, gradually shows a partial recovery, as one would have expected if it were an acute microbic infection. It is not unlikely that the beginning of the trouble is due to the absorptions of toxic bodies from the bowel which act at first as depressors of the functional activity of the pancreas and later the pancreatic islets undergo partial atrophy from disuse. Thus, if an excessive carbohydrate diet acts directly, as it undoubtedly does in those with weak pancreatic power or hereditary predisposition, in causing hyperglycæmia and glycosuria, its action is probably of the nature of an overstrain of the functional activity of the pancreas in providing the essential dextrose amboceptors: whilst, if it acts indirectly, it is probably best explained as due to the ill-effects of prolonged carbohydrate feeding on the digestive organs, leading to catarrh of the gastro-intestinal tract and consequent depression of the functional activity of the islets of Langerhans.

Exactly how diet acts in different individuals it is impossible to say; but, that there is a close relationship between the diet of the Hindu and glycosuria, no practising physician whom we have ever met doubts. In the series of papers already referred to, we have attempted to trace the gradual rise in the sugar content of the blood—as occurs in the well-fed leisured classes once they pass the energetic period of early manhood—this we have termed the pre-glycosuric stage of diabetes; and, as we have shown, it is accompanied by an increased fat content of the blood and a gradually increasing obesity; after a longer or shorter period of this pre-glycosuric stage with its low tolerance limits to carbohydrate ingestion, comes a stage of intermittent and transient glycosuria—the positive sugar reaction of the urine following, at first, the ingestion of sweet-meats, later, being present after a meal rich in carbohydrates; that is, the hyperglycæmia after sugar ingestion, or later, after carbohydrate ingestion, reaches a point beyond the threshold stimulus of the kidney for sugar excretion with the result that transient or intermittent glycosuria ensues. Still later, due to the decreasing power of synthesis of fat from dextrose and the decreasing power of tissue oxidation, the hyperglycæmia increases and the period of time intervening between each meal is insufficient to permit of the blood freeing itself from the excess dextrose before the advent of another relay of sugar from the intestinal tract. In this way the intermittent and transient

hyperglycæmia and glycosuria following each meal becomes constant and permanent.

If, however, the intervals between meals be prolonged suitably to the existing hyperglycæmia, the blood will still be able to free itself of the excess of sugar and the urine will again fall back to its previous condition, transient hyperglycæmia and glycosuria. *On this fact one of the great principles in the treatment of diabetes is based.*

#### THE TYPE OF DIABETES COMMONLY MET WITH IN INDIA.

As we have just said, in the ordinary glycosuric of the fat type if the intervals between meals be sufficiently prolonged, we obtain the exact reverse of what takes place during the onset and march of glycosuria; and, even in the most severe forms, with marked hyperglycæmia and urine heavily loaded with sugar, a few days of semi starvation is all that is necessary to decrease the sugar of the blood to normal and cause disappearance of the glucose from the urine.

Yet, under the ordinary conditions of Hindu life and dietary, these cases, of what may be regarded as alimentary glycosuria, will gradually progress into a condition showing all the ordinary complications of diabetes and terminating, as a rule, in much the same manner,—death from true diabetic coma being the outstanding exception. After a careful study of this condition for the last four years we are of the opinion that this type of glycosuria—whether intermittent or permanent, large or small in amount, easy or difficult to control—is a true diabetes from the beginning.

The disease corresponds in type to diabetes levis, in which glycosuria disappears on removal of carbohydrates from the diet. This is the type produced in dogs when 1/3th or 1/4th of the pancreas is left behind in partial extirpation of the organ. It differs from the severe forms of human diabetes in its long duration without apparently any very serious constitutional disturbances, and in its rather feeble tendency to pass into the category of diabetes gravis, in which glycosuria persists even on a diet containing no carbohydrates. It also differs very markedly from severe types of human diabetes in the comparative rarity of any signs of acidosis and the practical absence of true diabetic coma as a cause of death.

We have had under observation many cases that have been passing sugar constantly for years—some of them up to 20 to 30 years—without progressing into the more severe forms of the disease. What does take

place is that for the same quantity of carbohydrate ingested daily, the amount of sugar excreted increases slowly as time goes on. We have often seen patients who could produce old reports of urinary analyses which showed a gradual increase in the percentage of sugar, often for years. Of course this is a fallacious test as we did not know the quantity of urine voided on the dates the analyses were performed, but it shows the tendency to a gradual increase in the sugar excretion.

We have noted that the average educated Bengali clerk does not view his condition at all seriously until the urine shows over 5 per cent of sugar or until some complication, such as a carbuncle, frightens him.

Sooner or later in all cases, unless death has occurred from some intercurrent affection, a more serious state of affairs ensues. Not uncommonly quite early in the course of the disease albuminuria appears: wounds or sores heal badly: a slight knock, a slight illness, some trifle of which the patient has taken no notice, acts as the exciting agency and in a few hours or days the seemingly healthy glycosuric is *in extremis*.

The cause of death we hope to deal with exhaustively in a succeeding paper, but we have no desire to modify the statement made by us in 1916, that typical diabetic coma, as seen in severe human diabetes, practically never ends the scene. During the last four years, whilst dealing largely with diabetes, and for many years previously we have never seen a case of true diabetic coma in a Hindu suffering from the ordinary type of diabetes common in India. Coma does occur, but, as already stated and as we hope to prove in a later paper, the coma is uræmic, not diabetic.

The ordinary cause of death, however, is not coma but some complication or intercurrent disease: sepsis, gangrene, pneumonia, are common fatal complications.

The treatment of diabetes in India therefore resolves itself into the treatment of diabetes levis, or the milder forms of the disease met with in European countries. On the whole it is even a less serious and less fatal condition than the ordinary diabetes levis of Europe as it does not present the same tendency to progress, or to become complicated with severe acidosis and diabetic coma. We may state at once that we have not met with a single case, amongst the hundreds treated or seen by us, in which proper dietetic measures failed to clear the urine of sugar and keep it clear.

We propose to give some details of the early stages of the treatment of a few cases to illustrate the ease with which the excess of sugar in the blood and glycosuria can be eliminated. In the common type of diabetes met with in India, the glycosuria can be made to disappear in a few days, whatever may be the quantity of sugar that the patient is excreting. The degree of the glycosuria depends on the carbohydrate value of the diet, and once this type of patient begins to pass sugar in the urine, he can be made, by appropriate feeding, to eliminate large or small quantities of sugar, as desired.

*Cases illustrating the mild character of the type of Diabetes common in India.*

No. I.—This was the first case we treated after the enquiry had begun and, as will be seen, we very early got on to the methods of treatment now widely accepted as most satisfactory.

Date.	Quantity of urine. c.c.	Nitrogen of urine. grms.	Ammonia of urine. grms.	Sugar of blood. %	Sugar of urine. grms.	Diet. grms.
20-2-1915 ..	1075	4.5	1.52	.4	37	P- 65 C-214 F- 75
21-2-1915 ..	1188	4.96	.296		30.8	
22-2-1915 ..	1245	4.70	.42		56.0	
23-2-1915 ..	1471	5.70	.39		55.9	
24-2-1915 ..	1811	5.72	.41		<i>Nil</i>	P- 48 C- 88 F- 52
25-2-1915 ..	1528	4.90	.58	.113	<i>Nil</i>	
26-2-1915 ..	849	6.53	.28		<i>Nil</i>	

The fall to *nil* in this case was much more sudden than usually is the case; the cause doubtless being the fact that the second diet was well below the level of the patient's carbohydrate tolerance. There was no increase in the amount of ammonia excreted in the urine, and no increase in acetone. Di-acetic acid was absent throughout.

No. II.—European female, aged 50. Admitted for pain in the chest, cough, dyspepsia, fever, bronchitis: sugar, 5.25 per cent; albuminuria, copious; a large sloughing burn of the chest and both breasts from a hot-water bag.

Usual history of obesity, very fond of sweets, sugar, etc. The slough on chest increased and the wound showed no signs of healing



until the glycaemia was reduced to normal and the glycosuria had disappeared.

As this patient was very ill we were afraid at that stage—the second month—of the enquiry to reduce the diet suddenly. We, therefore, cut down the carbohydrates gradually. For our present purpose it will be sufficient to give here the results for the first diet on admission to hospital and the figures obtained when the diet was reduced below the patient's sugar tolerance.

Date.	Quantity of urine. c.c.	Nitrogen of urine. grms.	Ammonia of urine. grms.	Glucose of blood. %	Acetone of urine. grms.	Sugar of urine. grms.	Diet. grms.
22-2-1915 ..	1050	8.82	.56	.3	+	+	44.1
23-2-1915 ..	1200	11.16	.57	(about)	+	+	42
24-2-1915 ..	750	6.45	.54		+		46.5
25-2-1915 ..	850	5.77	.867		+		46.7
26-2-1915 ..	800	4.96	.446		+		32.0
27-2-1915 ..	950	5.90	.57		<i>Nil</i>		43.7
28-2-1915 ..	1250	6.50	1.06		<i>Nil</i>		52.5
From the 1st March the diet was reduced gradually until							
5-4-1915 ..	850	4.48	..	.16	.015	<i>Nil</i>	P- 14.7
6-4-1915 ..	600	4.25	..	(about)	.05	<i>Nil</i>	C- 35.6 F- 69
7-4-1915 ..	600	5.38	..		.06	<i>Nil</i>	P- 75.7
8-4-1915 ..	720	6.76	..		.048	<i>Nil</i>	C- 88.8
9-4-1915 ..	850	5.45	..		.11	<i>Nil</i>	F-116.2

From this date onwards to the middle of July the patient was under observation. There was no return of the sugar although the diet was increased gradually until the carbohydrate value was over 160 grammes daily. The patient has been seen at long intervals since and was keeping quite fit up to the last time we heard of her—about a year ago.

No. V.—Mahomedan male, aged 30. Sugar for one year. Severe constipation.

This case was also treated by a gradual reduction of the diet. Again, to illustrate our purpose at present, we give the results on the full hospital diet and on our restricted diet.

Date.	Quantity of urine, c.c.	Nitrogen of urine, grms.	Ammonia of urine, grms.	Acetone of urine, grms.	Glucose of blood, %	Glucose of urine, grms.	Diet, grms.
18-4-1915 ..	1670	10.34	.78	.032		138.6	P- 78.9
19-4-1915 ..	1520	8.80	.51	.013	.543	126.1	C- 516.8
20-4-1915 ..	1650	5.72	.44	<i>Nil</i>		125.4	F- 29.1
The diet was reduced gradually until							
5-5-1915 ..	1940	7.19	.25	.120		<i>Nil</i>	P- 26.1 C- 55.1 F- 49.5
6-5-1915 ..	1350	7.57	.32	.096		<i>Nil</i>	
7-5-1915 ..	1500	9.61	.45	.096	under .19	<i>Nil</i>	P- 106.4
8-5-1915 ..	1300	9.82	.41	.065		<i>Nil</i>	C- 56.0
9-5-1915 ..	900	7.37	.18	.054		<i>Nil</i>	F- 158.4
10-5-1915 ..	1650	11.28	.66	.011		<i>Nil</i>	
11-5-1915 ..	1600	8.48	.72	.015		<i>Nil</i>	

The diet was then gradually increased and the patient left hospital able to tolerate about 150 grammes of carbohydrate. The highly carbonaceous character of an ordinary dietary is exemplified by the figures given above for the analysis of the full hospital diet.

Other points exemplified by the results of the daily analyses are the low ammonia content and the absence of any signs of acidosis whilst on a restricted diet.

No. X.—Hindu male, aged 42. Clerk. He was very fat and flabby before the onset of glycosuria, which occurred three years before we saw him. He was accustomed to eat very largely of sweets, 1-2 lb. daily; no family history of diabetes. Weight in hospital, 113 lb.

Date.	Quantity of urine, c.c.	Nitrogen of urine, grms.	Acetone of urine, grms.	Glucose of blood, %	Glucose of urine, grms.	Diet, grms.
13-6-1915 ..	1400	5.25	.12		58.8	
14-6-1915 ..	2350	6.60	.07		47.0	P- 121.6
15-6-1915 ..	2200	5.96	.040		61.6	C- 330.1
16-6-1915 ..	2400	6.92	.028		48.0	F- 69.5
17-6-1915 ..	1850	3.49	.035		33.3	
18-6-1915 ..	2250	5.67	.027		27.0	
The diet was reduced below the patient's carbohydrate tolerance.						
19-6-1915 ..	2200	6.16	.048		<i>Nil</i>	
20-6-1915 ..	2900	6.61	.069		<i>Nil</i>	
21-6-1915 ..	2250	5.67	.065		3.6	P- 56.8
22-6-1915 ..	1950	3.99	.045	.153	<i>Nil</i>	C- 123.0
23-6-1915 ..	2830	7.71	.048		<i>Nil</i>	F- 102.4
24-6-1915 ..	3000	6.30	.075		<i>Nil</i>	

This patient never had any return of sugar, but left hospital before we had time to build up his tolerance beyond 150 grammes of carbohydrate per day.

Again we found little evidence of acidosis; on the other hand there are distinct signs of an increase in nitrogenous metabolism and in urinary excretion when patients are placed on a restricted diet.

No. XI.—Hindu male; no history: stayed in hospital 13 days only.

Date.	Quantity of urine. c.c.	Nitrogen of urine. grms.	Acetone of urine. grms.	Glucose of blood. %	Glucose of urine. grms.	Diet. grms.
2-7-1915 ..	3750	8.55	.011		225	P- 78.9
3-7-1915 ..	3750	18.37	<i>Nil</i>		270	C- 516.8
4-7-1915 ..	3750	7.42	.09	.493	232.5	F- 29.1
5-7-1915 ..	3750	7.61	.09		270	
6-7-1915 ..	1950	..	.063		107	P- 30.6
7-7-1915 ..	1100	4.19	.045		24.2	C- 77.5
8-7-1915 ..	1390	4.29	.062		<i>Nil</i>	F- 96.5

This patient exemplified very satisfactorily the very rapid fall that takes place in the excretion of sugar when the carbohydrate intake is reduced below the patient's tolerance.

The glycosuria falls from an average of 250 grammes per day to *nil* on the third day without any increase in the acetoneuria.

No. XVI.—Hindu male, aged 60. Eczema, polyuria, etc.

Date.	Quantity of urine. c.c.	Acetone of urine. grms.	Glucose of blood. %	Glucose of urine. grms.	Diet. grms.
18-11-1915 ..	1900	<i>Nil</i>	.40	77.4	
19-11-1915 ..	1050	<i>Nil</i>		39.9	P- 51.9
20-11-1915 ..	1100	<i>Nil</i>		50.6	C- 156.1
21-11-1915 ..	2200	<i>Nil</i>		70.1	F- 116.3
22-11-1915 ..	1850	<i>Nil</i>		61.0	
The carbohydrate was reduced; the protein and fat increased.					
23-11-1915 ..	2400	<i>Nil</i>		Trace	
24-11-1915 ..	1750	<i>Nil</i>		20.0	
25-11-1915 ..	1500	.034		<i>Nil</i>	P- 107.2
26-11-1915 ..	1700	.025		<i>Nil</i>	C- 55.1
27-11-1915 ..	1500	.028	.231	6.0	F- 153.9
28-11-1915 ..	2100	.049		<i>Nil</i>	
29-11-1915 ..	2600	<i>Nil</i>		<i>Nil</i>	
30-11-1915 ..	1800	<i>Nil</i>		<i>Nil</i>	

On the last two days some extra vegetables were given.  
Again we failed to find any evidence of acidosis.

No. XXVI.—Mahomedan male. Weight 76 lb. Glycosuria for seven months: no family history. Had lost a considerable amount of weight.

This looked at first to be a more severe type than usually met with; he reacted, however, very satisfactorily to treatment.

Date.	Quantity of urine. c.c.	Nitrogen of urine. grms.	Acetone of urine. grms.	Glucose of blood. %	Glucose of urine. grms.	Diet. grms.
25-4-1916..	2400	7.50	Nil		151.2	P- 60 C- 285.4 F- 124.4
26-4-1916..	2100	6.46	Nil		136.5	
27-4-1916..	2800	8.73	Nil		145.2	
28-4-1916..	2600	7.09	Nil	.43	163.8	
The type of diet was completely changed.						
29-4-1916..	1300	5.52	.113		56.4	P- 56.5 C- 122.3 F- 145.5
30-4-1916..	1200	5.20	.076		6.0	
1-5-1916..	1250	5.60	.090		Nil	
2-5-1916..	1300	4.73	.027		Nil	
3-5-1916..	1000	3.08	.029		Nil	
4-5-1916..	1300	4.23	.086		Nil	
5-5-1916..	850	2.79	.006	.20	Nil	
6-5-1916..	1350	4.72	.064		Nil	

On the last two days the diet was slightly increased by the addition of 4 oz. of green vegetables.

No. CCLIII.—Hindu male, aged 42. The patient had been very stout. Has had glycosuria to his knowledge for the last six months. He is now much emaciated—weight 82 lb.

Date.	Quantity of urine in c.c.	Glucose of urine in grms.	Diet in grms.
20-8-1917	2600	187.2	P- 52.2 C- 110.2 F- 106.0
21-8-1917	2550	170.0	
22-8-1917	1700	93.0	
23-8-1917	1900	98.8	
24-8-1917	1500	10.5	
25-8-1917	700	Nil	
26-8-1917	1800	Nil	

This patient was placed on a restricted diet on the day of admission to hospital: he became free from sugar on the fourth day. He was not reliable and later was caught stealing food.

No. CCXLVIII.—Hindu male, aged 30 years. The patient had cholera 18 months ago; soon afterwards polyuria set in and sugar was found in his urine. Is admitted almost blind from double cataract and is very emaciated. No albuminuria.

Date.	Quantity of urine. c.c.	Glucose of urine. grms.	Acetone of urine. grms.	Diet. grms.
2-8-1917	2800	134.4	<i>Nil</i>	P- 95.4 C- 412.5 F- 60.8
3-8-1917	2200	121.0	<i>Nil</i>	
4-8-1917	2400	132.0	<i>Nil</i>	
5-8-1917	2900	159.5	<i>Nil</i>	
6-8-1917	2000	96.0	<i>Nil</i>	
7-8-1917	1400	70.5	<i>Nil</i>	
8-8-1917	2300	126.5	<i>Nil</i>	
On the 8th the diet was changed.				
10-8-1917	900	19.8	<i>Nil</i>	P- 58.2 C- 99.0 F- 107.0
11-8-1917	1000	<i>Nil</i>	<i>Nil</i>	
12-8-1917	1300	<i>Nil</i>	<i>Nil</i>	
13-8-1917	1500	<i>Nil</i>	<i>Nil</i>	

This patient remained in hospital for nearly three months and only on one occasion showed a trace of sugar. The diet was increased eventually to over 200 grammes of carbohydrates.

No. LXX.—European male, aged 47. Very stout, weight over 280 lb. six months ago. Has led a very active life. He has had glycosuria for five years and suffered during that time with carbuncle, boils and neuritis. He was circumcised for balanitis and had a very severe infection after, the wound taking a long time to heal. Father suffered from diabetes.

Date.	Quantity of urine. c.c.	Sugar of blood. %	Sugar of urine. grms.	Diet. grms.
9-3-1917	( ? )	32	7	P- 53.5 C- 76.6 F- 57.0
10-3-1917	1450	..	60.9	
11-3-1917	850	..	11.1	
12-3-1917	800	..	11.2	
13-3-1917	800	23	13.6	
14-3-1917	850	..	Trace	P- 24.42 C- 44.4 F- 26.5
15-3-1917	700	..	Trace	
16-3-1917	800	..	<i>Nil</i>	
18-3-1917	750	993	<i>Nil</i>	

This patient was on ten days' casual leave and spent it in hospital in order to learn the method of treatment. He dieted himself after returning to duty and succeeded in reducing his weight about 100 lb. His urine remained free from sugar, until he left India some six months later. His health in the meantime improved. Recently news from this patient has been received and we understand that he is doing well. His weight has decreased considerably, but his urine is sugar-free and he is carrying out his very arduous duties with full energy.

The ten cases given above are quite sufficient to demonstrate the comparative ease with which the glycosuria met with in all classes of individuals in India can be controlled. Large numbers of patients have been treated and they practically all give similar results.

In a few cases we declined to diet patients on account of their very serious condition on admission—such as those dying of phthisis, pneumonia, gangrene, etc.

It may, therefore, be accepted that the form of diabetes common in India is diabetes levis of a comparatively slowly progressing type.

It may be well here to refer to a result which made its appearance in the very earliest of our cases—as shown above—and which had a very important influence in determining the method of treatment we afterwards adopted. This was that a reduction of the carbohydrate of the diet below the patients' tolerance point never caused us any anxiety on the score of acidosis. A trifling amount of acetone appeared in some cases, but never at any time during the last four years have we obtained a positive reaction for diacetic acid in an Indian treated by us.

In the early days of 1915 this was a matter of the very greatest moment, as acidosis leading to diabetic coma was theoretically the great danger we feared. Often have we gone to the hospital throughout the day and night to see for ourselves the condition of patients who were being treated by a restriction of diet. Never once have we seen the least sign of coma from that cause, and we have never been forced to administer alkalis on account of signs of increasing acidosis.

This had a most important bearing on the problem of the type of the disease we were called on to treat, and the fact that there was no cause to fear acidosis or coma gave us courage to retain our patients for considerable periods of time on diets of a carbohydrate value considerably less than the individuals' tolerance. It became evident before long that the diet could be increased in all its proximate

principles without glycosuria returning or hyperglycæmia reappearing. Thus the line of treatment, which we have followed seemingly with great success since February 1915, was made evident to us by the results we obtained from analyses of the blood and urine, and from the indication afforded by the absence of acetone and di-acetic acid in the urine of patients undergoing treatment by severe restriction of the carbohydrate value of their diet. This is a matter to which we shall have to return later, but we may refer the reader to Charts I and III which illustrate the principle to which we allude. These charts deal with two of our earlier cases, and the charts were shown and the method of treatment explained at the Annual Meeting of the Reunion of Graduates of the Medical College, Calcutta, in 1915.

The principle in the treatment of the type of diabetes seen commonly in India, which gradually became evolved as we found that the dietary of a patient could be increased without any return of glycosuria, was the principle of the re-education of the diabetic to tolerate gradually increasing quantities of carbohydrates.

It will be evident from the dates given when each case was treated that the principle of treatment herein outlined had been already worked out in considerable detail before the publication of Allen's monumental work.

From February 1915 up to August 1916, when we first received Allen's book, our records show that we had treated a considerable number of patients on the lines of clearing the blood of hyperglycæmia and the urine of sugar; and then, by a gradual increase in the different element of the diet, slowly building up the patients' carbohydrate tolerance by a re-education of his tissues to utilize carbohydrates.

After the publication of Allen's method of treatment, which essentially consists in an initial period of starvation followed by a slow building-up process, our own method had proved so satisfactory that we have never, except as an experiment, made use of the complete starvation which Allen advocates. The reason why complete starvation in this country is unnecessary is doubtless because the type of diabetes is so mild.

We may be permitted to state that the principle of treatment herein advocated has been evolved entirely independently of the splendid research work done by Allen on animals and published in his "*Glycosuria and Diabetes*." The evolution of the treatment entirely depended on the absence of all signs of acidosis. If acetone or di-acetic acid had been



present in any quantities we should have probably increased the carbohydrate of the diet beyond the level of the patients' tolerance and thus no progress could have been made. That is, we should have had to remain content with the old method of treatment, restricting the carbohydrates and obtaining the necessary potential energy from fat and protein.

#### THE EVOLUTION OF THE METHOD OF TREATMENT OF DIABETES IN INDIA.

In 1913 one of us began the treatment of diabetes, by dieting, on the principle which later has worked out to be successful. The idea underlying the method was to reduce the diet, or at least the carbohydrates, below the level at which the patient under treatment excreted sugar; then, after a week or so, slowly increase the diet in the hope that the patient's carbohydrate tolerance would improve—on the analogy that overstrain of an organ will cause a weakening of its function whilst rest will tend to strengthen function.

The experimental results were seemingly corroborating our forecast when, unfortunately, the serious illness of the officer in charge of the work brought the research to an end. Nothing more was done until the present enquiry opened in January 1915 when we took up the treatment at the point where it was left off in July 1913.

As already stated, in those early days we were imbued with the great fear of acidosis and diabetic coma, and with the danger of doing anything which might increase acidosis or precipitate an attack of coma by too enthusiastically cutting down the carbohydrate value of the diet. Therefore, from the very beginning of the treatment we decided to carry out a complete quantitative analysis daily of the urine, so that any signs of acidosis might be brought to light at once and steps taken to forestall trouble. With this object in view a careful record of the daily analyses of the urine was made under the following headings:

1. Quantity excreted.	5. Ammonia.
2. Total nitrogen.	6. Acetone.
3. Urea.	7. Di-acetic acid.
4. Uric acid.	8. Sugar excreted.

The dietaries were changed at intervals and their values in proximate principles noted. In addition, the percentage of sugar in the blood was estimated as often as it was thought necessary.

It was very soon quite evident, from the results of the estimation of ammonia, acetone and di-acetic acid, that the great theoretical danger to be avoided was non-existent. Instead of Indian diabetics showing an increase to any serious extent in the signs of acidosis, we found, on the contrary, that even severe carbohydrate restriction never caused di-acetic acid to appear; that acetone, if present, was trifling in amount and that, after a few days of a restricted diet, it disappeared; and finally, after a period of anxiety, we were forced to the conclusion—absolutely opposed to the opinion held in those days—that coma was not to be feared.

This finding gave us courage to pursue our plan of treatment with tenacity of purpose, and not to fear the appearance of a little acetone when the diet was cut down.

The great trouble we found in the treatment of diabetics was not acidosis, or any tendency thereto, but the difficulty of preventing patients from obtaining extra food by stealing or by some other means. When a reliable patient was treated, no great difficulty was experienced in controlling the glycosuria and in gradually building up his carbohydrate tolerance, and thus re-educating his system to utilize glucose. This could be accomplished and patients built up to a level of carbohydrate metabolism that permitted of a fairly liberal daily diet.

We were fortunate in finding, early in the enquiry, several patients whose word could be relied on. The results derived from observations on these cases placed us in a position to be able to speak definitely with regard to the success of the method of treatment, and also gave us confidence in assuring patients that, with a little patience and forbearance on their part, a great amelioration of their condition was certain and a comparative cure would be most probable.

Many different food-materials were experimented with and different so-called cures investigated. Thus the "oat-meal cure," "rice cure," "soya bean cure" were all tried and abandoned as useless. Several aids to the dietetic treatment were tried, some with a certain amount of success—at least temporarily. Extract of liver does appear to have an influence on lessening the excretion of sugar. Trypsogen also sometimes for a short period decreases the excretion of sugar on a constant diet.

Gradually, however, we gave up all such experiments and placed our faith in a proper method of dieting. This we have no cause to regret, as hundreds of diabetics at the present time are carrying out the

treatment, on the principle outlined above, with success. Complications clear up; gangrene, carbuncles, boils, heal; albuminuria, so long as there is no organic renal disease, disappears; and, what is most important of all in India, the tendency to uræmia—the real danger of the diabetes of this country, so far as coma is concerned—fades away.

We shall now proceed to give in a certain amount of detail the result of the observations made on the urine and blood of one of the first cases treated by a re-building of the patient's carbohydrate tolerance.

We have already made use of the results of a few days' treatment of this case to show how easily glycosuria can be controlled; we shall now give details of the treatment adopted and the results achieved.

It would take up a great deal of space to give daily analyses; we have therefore pooled the figures for the different dietary periods and shown them in the form of daily averages. In addition Falta's coefficient for sugar excretion has been calculated and given in a separate column. This is obtained by estimating the percentage of the carbohydrates in the total food that is lost to the system and is calculated from the formula :

$$\frac{\text{Quantity of sugar excreted in urine}}{\text{Nitrogen of urine} \div 3.65 \div \text{carbohydrate value of diet}} \times 100$$

Diabetic No. II.—European female, aged 50 years. Admitted for pain, cough, dyspnoea, bronchitis, glycosuria 5.25 per cent. copious albuminuria, sloughing burn of the chest and both breasts due to a hot-water bag.

There was a suspicious history of glycosuria some years ago. The patient is married, has had no children. She lives on a European type of diet but is very fond of sweets. She is stout, flabby, and anæmic. Looks very ill.

GRMS.

70

65

60

55

50

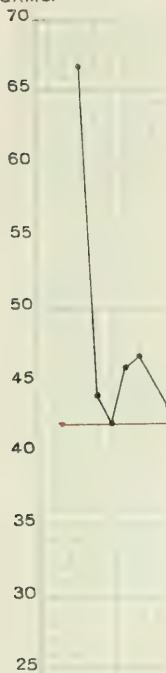
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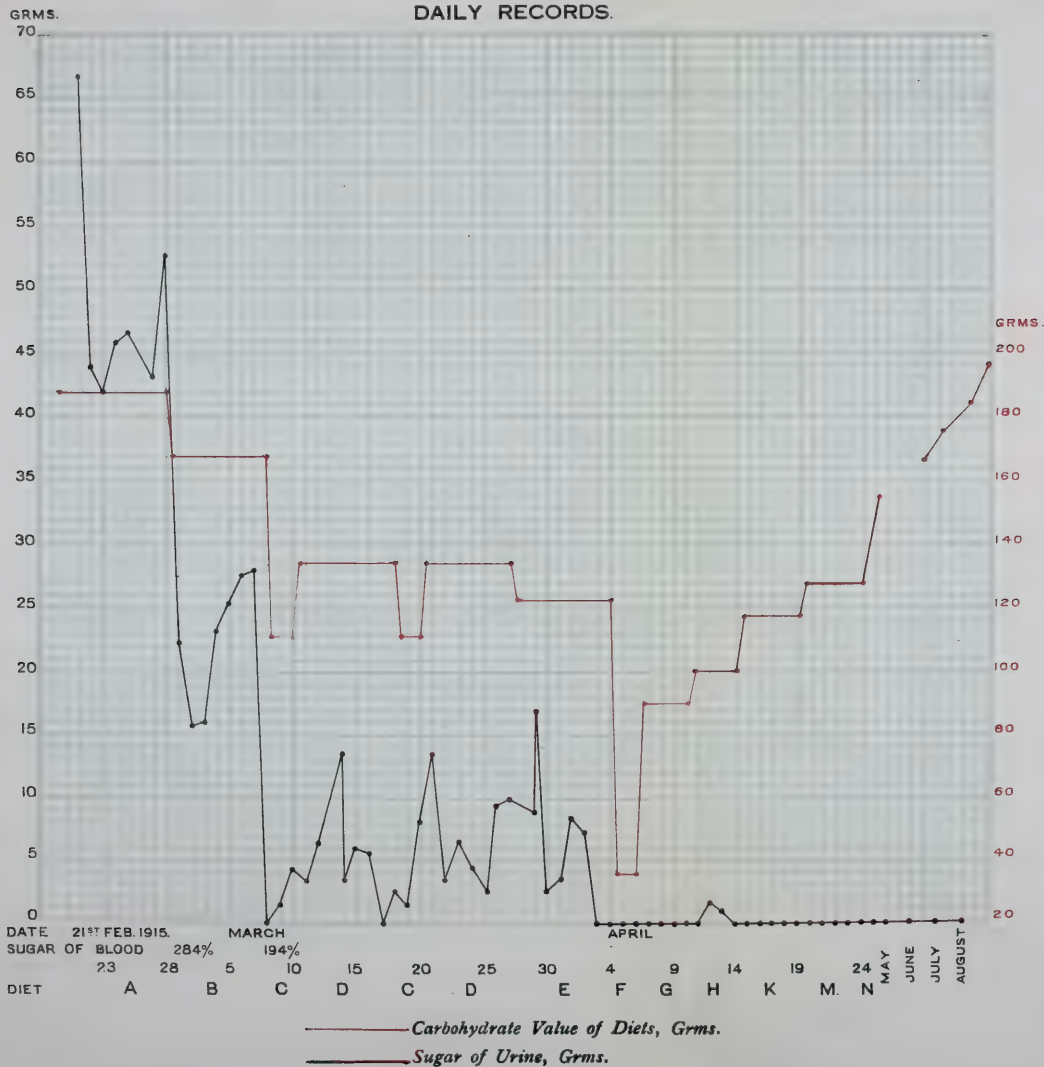
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# CHART I.

DIABETIC No. II

DAILY RECORDS.



Date.	Quantity of urine, c. c.	Nitrogen of urine, grms.	Uric acid, grms.	Ammonia, grms.	Acetone of urine, grms.	Glucose of blood, %	Glucose of urine, grms.	Falta's coeffi- cient, %	Diet	Grms.
22-2-1915 to 28-2-1915	980	7.08	.47	.67	+	.3 about	44	20.6	Milk 1 1/2 srs.* Bread 8 oz. Butter 1 oz.	P- 78.3 C- 187.7 F- 95.5
1-3-1915 to 8-3-1915	1360	6.00	.49	.67	.065	.284	23.2	12.2	Milk 1 sr. Eggs 6 oz. Vegts. 12 oz. (Oatmeal 5 oz.)	P- 77.12 C- 167.6 F- 123.8
9-3-1915 to 10-3-1915	1200	6.26	.46	.70	.073	.194	Trace	0.5	Milk 1 sr. Vegts. 24 oz. Eggs 8 oz. Butter 2 oz.	P- 68.2 C- 110.2 F- 126.0
11-3-1915 to 18-3-1915	1306	6.37	.465	.70	.051	..	7.1	4.5	Milk 1 sr. Eggs 8 oz. Vegts. 16 oz. Oatmeal 24 oz. Butter 2 1/2 oz.	P- 73.5 C- 133.3 F- 143.4
19-3-1915 to 20-3-1915	1100	5.94	.455	..	.0245	..	Trace	0.5	Milk 1 sr. Vegts. 24 oz. Butter 2 oz. Eggs 8 oz.	P- 68.2 C- 110.2 F- 126.0
21-3-1915 to 27-3-1915	1550	6.17	.42	.64	.0363	..	5.84	3.7	Milk 1 sr. Vegts. 12 oz. Eggs 6 oz. Oatmeal 24 oz. Butter 2 oz. Fish 4 oz.	P- 87. C- 122.1 F- 115.0
28-3-1915 to 4-4-1915	1365	7.38	.43	.92	.0336	..	7.52	5.0		

\* 1 Seer (sr.)=32 oz.

Date.	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid of urine, grms.	Ammonia, grms.	Acetone, grms.	Glucose of blood, %	Glucose of urine, grms.	Fat's coeff. cent. %	Diet.	Grms.
5-4-1915 to 6-4-1915	725	4.54	445	..	0.025	..	Nil	Nil	Vegts. 12 oz. Eggs 4 Butter 2 oz. Oranges 2	P. 117 C. 35.6 F. 69.0
7-4-1915 to 10-4-1915	740	5.83	410	..	0.044	..	Nil	Nil	Milk 1 <sup>1</sup> pt. Eggs 6 Bacon 2 oz. G. Soya beans 3 oz. Butter 2 oz. Orange 1	P. 80.7 C. 88.8 F. 120.2
11-4-1915 to 14-4-1915	760	6.21	448	0.72	0.042	..	Nil	Nil	Diet G plus H Two chops Potatoes 2 oz.	P. 86.0 C. 100 F. 125
15-4-1915 to 19-4-1915	800	..	..	..	Nil	..	Nil	Nil	Do plus K Bread 1 oz.	P. 88.5 C. 117.5 F. 127.0
(Equal quantities of chop, fish and chicken interchangeable.)										
20-4-1915 to 24-4-1915	800	..	..	..	Nil	..	Nil	Nil	Milk 1 pt.* Vegts. 16 oz. Butter 2 oz. M. Chicken 8 oz. Potatoes 2 oz. Eggs 6 Bread 2 oz.	P. 95.4 C. 127.8 F. 124.2
25-4-1915 to 29-4-1915	(?)	..	..	..	Nil	..	Nil	Nil	Do plus N Legumis 2 oz.	P. 108 C. 134 F. 127

\* 1 Seer (sr.) = 32 oz.



Patient left hospital able to tolerate between 150 and 160 grammes of carbohydrate. Di-acetic acid was absent throughout.

Returned.

15-5-1915 ..	} No glycosuria .. Diet estimated to be worth ..	160—200 grms. of carbohydrate
15-6-1915 ..		
24-7-1915 ..		
30-8-1915 ..		

Later for two years the urine was examined and the patient seen occasionally: there was no return of the sugar: the lady was living on a fairly liberal carbohydrate diet, sufficient for her bodily requirements.

This patient was very seriously ill on admission to hospital so that no drastic cutting down of the diet was attempted. The sloughing burn of the chest showed little sign of healing until the glycosuria had disappeared: it then cleaned up and healed rapidly. This patient, so far as our conception of the disease was concerned, we regarded as a test case, as the copious albuminuria complicated her condition very seriously. In addition the bronchitis and tendency to toxæmia and cyanosis called for very careful treatment. The effects of treatment, as recorded above for the different diets, were most satisfactory and gave us great confidence in the treatment of similar types of cases, with or without complications.

The salient features brought out by the method of treatment adopted in this case are:—

- (1) The increase in the quantity of urine excreted. This was important as we feared uræmia more than diabetic coma.
- (2) The absence of any signs of acidosis. Acetone eventually disappeared completely when the patient's tolerance to carbohydrates began to increase. The ammonia content of the urine remained fairly constant.
- (3) The patient's tolerance lay between 80 and 100 grms. of carbohydrate daily.
- (4) By a gradual increase in the diet, all proximate principles were increased and the carbohydrate tolerance was built up practically 100 per cent.
- (5) The great improvement that took place in the general condition of the patient as soon as the hyperglycæmia and glycosuria were controlled.

Diabetic No. IV.—Mrs. J., European female, aged 47. Married. Mother died of diabetes. Patient is the mother of three healthy children. She is accustomed to a rich diet; curry and rice twice daily; is very fond of sweets.

Two years ago had boils and great irritation around the genitals—these signs disappeared after two months.

In January 1915 she got a similar attack with frequent micturition. The boils came out all over the body. In March, sugar—4 per cent—was discovered in the urine and she came to hospital for treatment.

The patient is strong and healthy looking. There are no signs of toxæmia or cachexia. She is fat and anæmic. Has lost weight recently.

This was a very different type of patient to that recorded above; we felt no misgivings in cutting down the diet at once, as soon as some idea of her condition had been obtained from the effects of a test diet.

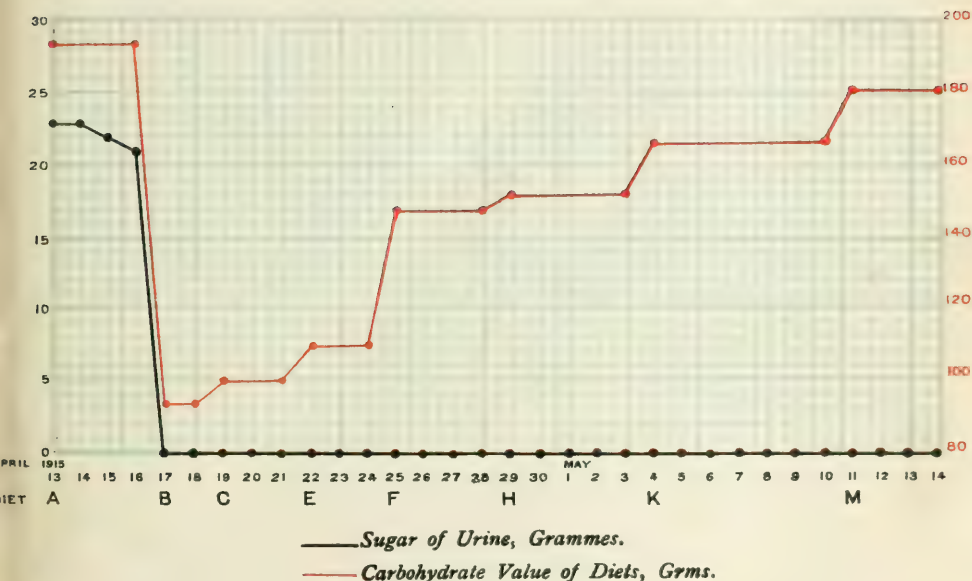
Owing to the eczematous condition of the external genitals there was difficulty in collecting the urine but we obtained enough for our purpose.

Date.	Quantity of urine saved. c.c.	Acetone of urine. grms.	Sugar of urine. grms.	Diet.	Grms.
13-4-1915..	700 ?	·032	23·3	Milk 1½ sr.	
14-4-1915..	700 ?	·062	23·3	Bread 8 oz.	P = 94·9
15-4-1915..	700 ?	Nil	22·4	Butter 2 oz.	C = 187·7
16-4-1915..	500 ?	Nil	21·5	Fish 6 oz.	F = 123·6
				Eggs 2	
17-4-1915..	300 ?	Nil	Nil	Milk sr.	
18-4-1915..	400 ?	Nil	Nil	Vegts. 116 oz.	P = 60·7
				Butter 2 oz.	C = 87·8
				Bacon 2 oz.	F = 130·0
				Eggs 4	
19-4-1915..	700	Trace	Nil		P = 61·9
20-4-1915..	850	Trace	Nil	Do. plus	C = 100·0
21-4-1915..	1200	Trace	Nil	Potatoes 2 oz.	F = 131·2
22-4-1915..	1150	Nil	Nil		P = 63·7
23-4-1915..	1200	Nil	Nil	Do. plus	C = 115·6
24-4-1915..	1350	Nil	Nil	Potatoes 3 oz.	F = 133·6
25-4-1915..	850	Nil	Nil	Milk 1 oz.	
26-4-1915..	1850	Nil	Nil	Vegts. 16 oz.	
27-4-1915..	1420	Nil	Nil	Butter 2½ oz.	P = 84·4
28-4-1915..	1200	Nil	Nil	Bacon 2 oz.	C = 144·1
				Chops 4 oz.	F = 135·3
				Potatoes 3 oz.	
				Bread 3 oz.	

# CHART II.

DIABETIC No. IV

DAILY RECORDS.





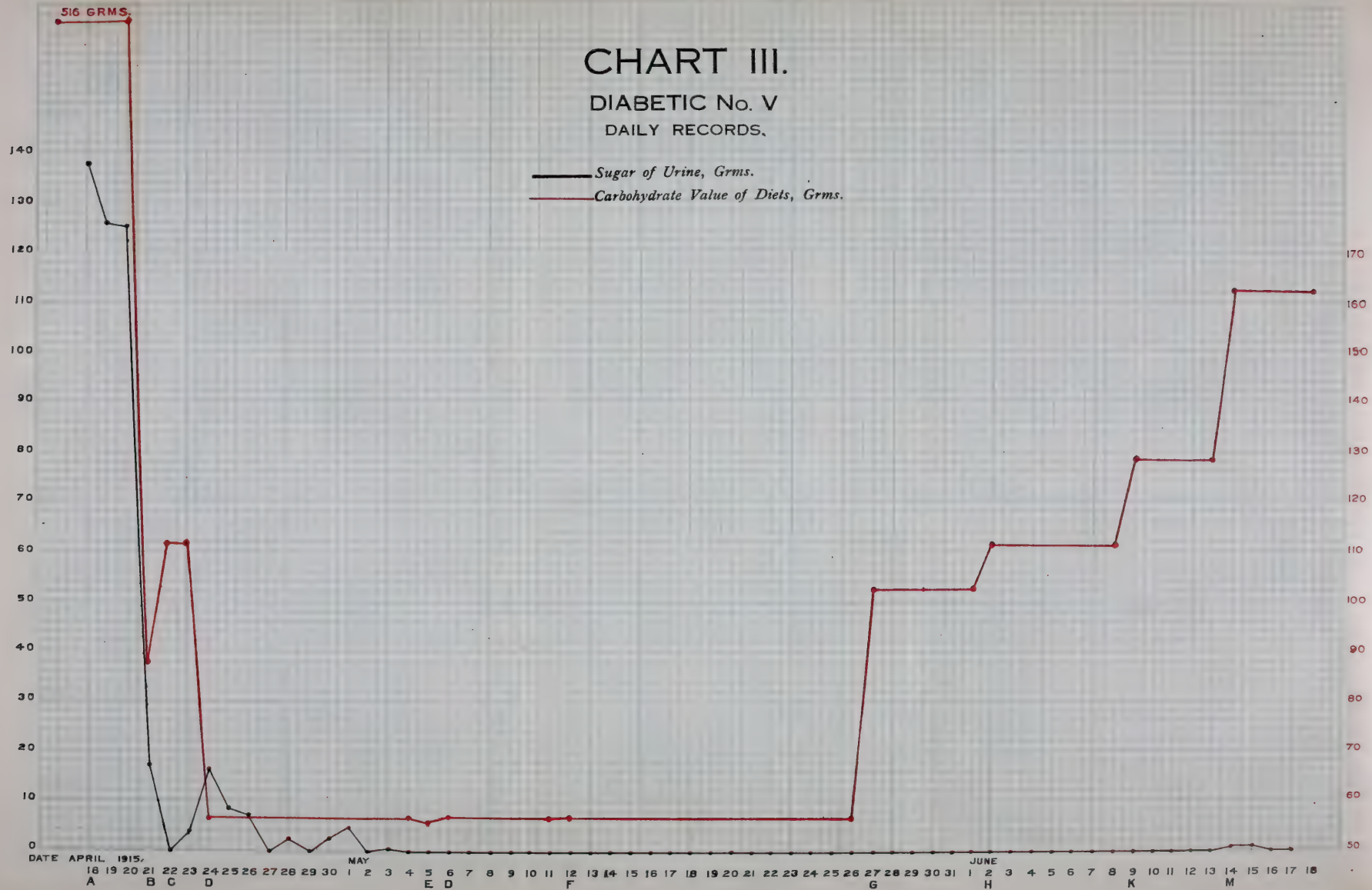


# CHART III.

DIABETIC No. V

DAILY RECORDS.

— Sugar of Urine, Grms.  
— Carbohydrate Value of Diets, Grms.



Date			Diet.	Urn.
29-4-1915	No glycosuria	H	Bread plus	{ P=85.7
to			Potatoes 2 oz.	{ C=146.0
3-5-1915				{ F=136.2
4-5-1915	Ditto	K	Do plus	{ P=88.2
to			Bread 1 oz.	{ C=163.4
10-5-1915				{ F=138.0
11-5-1915	Ditto	M	Do plus	{ P=90
to			Bread 1 oz.	{ C=180
14-5-1915				{ F=140

The patient left hospital on the 15th April, 1915, completely free from eczema, boils, etc., and with her carbohydrate tolerance built up to 180 grammes daily.

This was a comparatively simple case and the tolerance could have been further increased, but the patient was anxious to go home. She dieted herself and sent the urine some months afterwards which was free from sugar. We do not know however what the diet was.

Diabetic No. V.—B. K., Mahomedan male, aged 30. Illness of one year's duration. Accustomed to ordinary Mahomedan type of dietary. He suffered greatly from constipation and states that the signs of diabetes came on suddenly after taking a purgative. Weight 79 lb.

Chart No. III gives this case in detail, so we shall content ourselves with stating the average figures for the different dietary periods.

(This chart and the methods of treatment adopted were demonstrated at the Annual Meeting of the Reunion of Graduates of the Medical College, Calcutta, in 1915.)

Each day the urine was completely analysed and the total nitrogen, uric acid, ammonia, acetone and sugar estimated.

The presence or absence of di-acetic acid was noted. The figures given, therefore, represent about 60 analyses under each different heading. Some difficulty was experienced in preventing the urine from decomposing and thus obscuring the real excretion of ammonia; however, we were able to obtain sufficient evidence to prove that there was no increase in the ammonium compound and that, therefore, there was no increase in acid elimination, *i.e.*, no acidosis. Further evidence of this was afforded by the estimation of acetone and the prevailing absence of di-acetic acid.

An analysis of the results obtained by dietetic methods in this case is very interesting.

The carbohydrate values of the diets B, C, and D, from 21st April, 1915, to 11th May, 1915, taking into account the carbohydrates derived from protein metabolism—nitrogen of the urine multiplied by 3.65—are 110, 134, and 88 grammes respectively.



Date	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid, grms.	Ammonia, grms.	Acetone of urine, grms.	Glucose of blood, %	Glucose of urine, grms.	Diet.	Grms.
18-4-1915 to 20-4-1915	{ .. }	8.28	.46	.57	.0225	.543	132.6	A { Full hospital diet including Rice 20 oz.	{ P=78.9 C=516.8 F=29.1
21-4-1915	..	6.16	.212	.374	.052	..	17.6	B { Milk 1 sr.* Vegets. 16 oz. Butter 2 oz.	{ P=47.7 C=87.8 F=102.9
22-4-1915 to 23-4-1915	{ .. }	6.13	.342	.562	.027	..	10.8	C { Milk 1 sr. Fish 4 oz. Eggs 6 oz. Vegets. 16 oz. Oatmeal 2 1/2 oz. Butter 2 1/2 oz.	{ P=58.5 C=111.8 F=115.9
24-4-1915 to 4-5-1915	{ .. }	8.82	.447	.449	.076	.49	2.7	D { Fish 4 oz. Vegets. 20 oz. Eggs 6 oz. Butter 2 1/2 oz. Curds 16 oz.	{ P=106.4 C=56.0 F=154.9
5-5-1915	..	7.19	.504	.25	.12	..	Nil	E { Milk 1 sr. Vegets. 12 oz. Butter 1 oz.	{ P=26.1 C=55.1 F=49.5
6-5-1915 to 11-5-1915	{ .. }	9.02	.497	.46	.057	..	Nil	D Diet D	

\* 100r (sr.) = 32 oz.

Date.	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid, grms.	Ammonia, grms.	Acetone of urine, grms.	Glucose of blood, %	Glucose of urine, grms.	Diet.	Grms.
12-5-1915 to 26-5-1915	1310	13.37	.75	.344	.002	.15	Nil	Vegts. 20 oz. Butter 2½ oz. Eggs 6 Fish 4 oz. Curds 16 oz. Sunato- 2 oz. gen.	Diet D plus Sunatogen 2 oz. Carbohydrate 76 grms. (about)
27-5-1915 to 1-6-1915	1345	8.58	.573	....	Trace	..	Nil	Milk ½ st.* Fish 4 oz. Eggs 6 oz. Vegts. 16 oz. Oatmeal 2 oz. Butter 2½ oz.	P—56.5 C—102.7 F—115.5
2-6-1915 to 8-6-1915	1150	7.66	.55	..	Nil	..	Nil	Diet G plus Oatmeal ½ oz.	P—58.4 C—111.8 F—116.3
9-6-1915 to 13-6-1915	1530	6.26	.517	..	Nil	.17	Nil	Do. plus Bread 1 oz.	P—61.0 C—128.0 F—117.6
14-6-1915 to 18-6-1915	1550	..	..	..	..	.199	Trace	Do. plus Bread 2 oz.	P—63.8 C—162.8 F—119.8

\* SEET (ST.) = 32 oz.

The patient was anxious to leave, so 2 oz. of bread extra were tried but it was too sudden an increase and a trace of sugar appeared—the hyperglycemia returning.

During this fortnight the patient continued to pass sugar. Even on diet D, containing only 56 grammes of carbohydrate derived entirely from green vegetables and 32 grammes from protein metabolism or 88 grammes of carbohydrates, the glycosuria remained during the eleven days of the diet. A complete change of the character of the diet and a decrease of the vegetables by 4 oz. caused the sugar to disappear. The patient's tolerance was therefore about 55 grammes of carbohydrate plus 26 grammes from protein metabolism, or a total carbohydrate tolerance of 81·3 grammes.

From that date, after six days of low carbohydrate ingestion to clear the blood of excess sugar, the diet was gradually increased. The patient gradually, during the succeeding six weeks, regained his power of utilizing carbohydrates and in the end, before he insisted on leaving hospital, he was able to tolerate practically three times the quantity of carbohydrates in his diet that previously caused glycosuria. His total carbohydrate tolerance, including the carbohydrates from protein breakdown, improved well over 100 per cent.

In this case, as in those previously treated on these lines, we were encouraged to proceed by the absence of any signs of acidosis. The patients also admitted, despite the somewhat meagre diet, that they felt better and stronger than before treatment. In fact the trouble often was to get them to remain in hospital, once they began to feel the benefits of treatment.

Diabetic No. IX.—W. L., European male, aged 65 years. This patient had been a fine strong man and had led a healthy open air life. He had spent most of the early years of his life at sea.

He was admitted to the surgical wards for gangrene of the big toe and a septic foot. Three weeks previously he had sustained a slight injury to the toe and the inflammation spread upwards. Eventually, four toes and the adjoining parts of the right foot had to be taken off and the foot opened up to the heel. The wounds healed up rapidly as soon as the hyperglycæmia and glycosuria were got under control.

The patient was very ill on admission and we were not at the time—May 1915—sufficiently confident of our position to care to risk the onset of coma by a sudden withdrawal of carbohydrates. The patient was, therefore, treated very carefully and the diet restricted in a gradual manner. There was also some albuminuria present which did not tend to increase our courage or counsel drastic treatment.

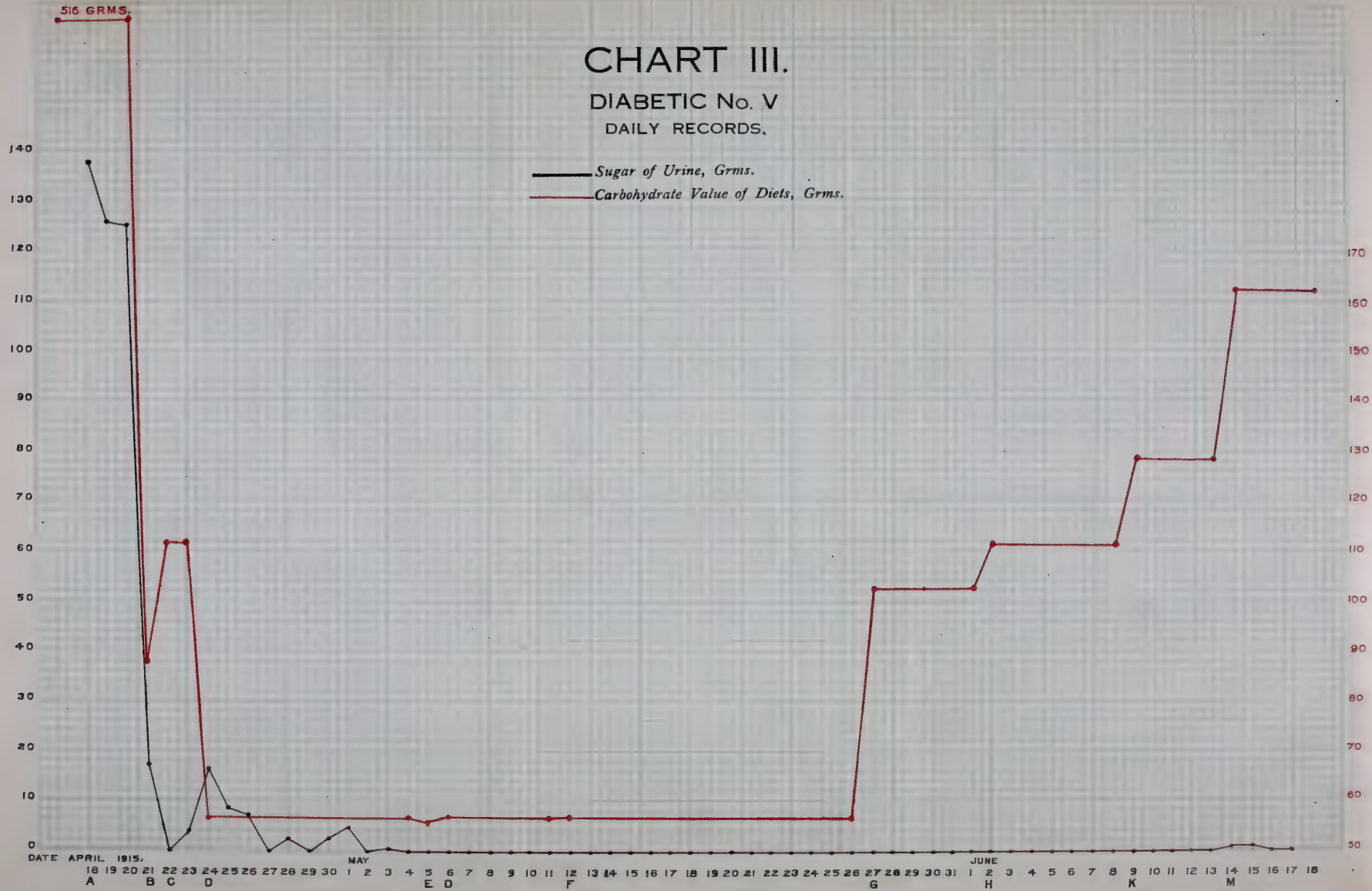


# CHART III.

DIABETIC No. V

DAILY RECORDS.

— Sugar of Urine, Grms.  
— Carbohydrate Value of Diets, Grms.



So far as the diabetes was concerned the patient did exceedingly well and the manner in which he reacted to dietetic treatment and regained his carbohydrate tolerance left almost nothing to be desired. His general condition, however, was never very satisfactory: he never lost the anæmic, toxæmic or cachectic appearance that was present on admission. Later, long after the hyperglycæmia and glycosuria had disappeared, the albuminuria began to increase, slight œdema appeared, the patient became restless and gradually sank into coma and died. Death was due to typical uræmic coma preceded by a gradual suppression of urine.

There were absolutely no signs of acidosis at any time, either in the urine or the breath. It was the gradual sinking into uræmic coma of this patient that finally confirmed our previous suspicions that the coma seen in India is very seldom diabetic, but uræmic.

The patient was admitted on the 15th of May, 1915, and died on the 9th August, 1915. Daily records of the urinary analyses were kept under the usual headings. It would take up too much space to give these in full detail; we shall therefore only publish the averages of the figures obtained for the different dietary periods. From the first week in July 1915 the decrease in the quantity of urine excreted daily began to appear: it rapidly got worse towards the end of July and the patient began to have attacks of vomiting, twitchings, and restlessness. These signs became more marked, he became semi-conscious, sank into coma and died.

Date.	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid, grms.	Acetone of urine, grms.	Glucose of blood, %	Sugar of urine, grms.	Diet.	Grms.
15-5-1915 to 18-5-1915	1320	10.47	.553	.0372	.28	34.4	Milk 1½ sr.* Eggs 2 Custard 1 Beet tea 4 oz. Bread 6 oz. Butter 1 oz.	P- 83.3 C- 166.9 F- 99.7
19-5-1915 to 2-6-1915	1375	9.26	.53	.002	.22	28.3	Milk 1½ sr. Eggs 5 Chicken jug 4 oz. Bread 4 oz. Vegts. 8 oz. Butter 2 oz. Oranges 2	P- 89.7 C- 148.7 F- 134.0
3-6-1915 to 12-6-1915	1345	7.08	.467	.048	..	23.6	Do. plus Trypso- gen 16 tablets daily.	
13-6-1915 to 23-6-1915	1365	5.31	.43	.059	17	3.5 (and after a few days)	Milk 1 sr. Eggs 6 Butter 2½ oz. Bread 2 oz. Vegts. 8 oz. Chicken 6 oz. Curds 6 oz. Peaches 4	P- 93.6 C- 96.2 F- 154.7

\* 1 Seer (sr.) = 32 oz.



Date.	Quantity of urine. c.c.	Nitrogen of urine. grms.	Uric acid. grms.	Acetone urine. grms.	Glucose of blood. %	Sugar of urine. grms.	Diet.	Grms.
24-6-1915 to 29-6-1915	1420	7.38	.22	.101	....	Nil	D { Diet C plus Potatoes 2 oz.	P-94.8 C-107.3 F-155.3
30-6-1915 to 4-7-1915	985	3.87	.271	.032	....	Nil	E { Diet C plus Potatoes 2 oz. Bread 1 oz.	P-97.3 C-124.4 F-156.5
5-7-1915 to 13-7-1915	885	4.00	.285	.030	.2	Nil	F { Diet C plus Potatoes 2 oz. Bread 2 oz.	P-99.4 C-141.2 F-157.7
The balance of the diet completely changed as the sugar of the blood was rising.								
14-7-1915 to 22-7-1915	880	3.70	.31	.042	.15	Nil	H { Milk 1 sr.* Potatoes 4 oz. Onions 1 oz. Butter 2 oz. Chicken 8½ oz. Vegts. 8 oz. Bread 3 oz.	P-65.3 C-138.5 F-120.3
The building-up continues but elimination fails.								
23-7-1915 to 26-7-1915	520	3.02	.37	.017	....	Nil	K { Do. plus Rice 1 oz.	P-67.2 C-155.8 F-121.0
27-7-1915 to 1-8-1915	156	....	....	Slight trace	....	Nil	M { Milk 1 sr. Bread 5 oz. Butter 2½ oz. (Chicken jug 2 oz.) Rice 2 oz.	P-54.5 C-173.3 F-110.6

1 Seer (sr.) = 32 oz.

From this date uræmic signs set in and the diet was largely milk until death.

2-8-1915	..	..	250	No sugar. No di-acetic acid. Slight trace of acetone. Abundant albumen.
3-8-1915	..	..	150	
4-8-1915	..	..	200	
5-8-1915	..	..	150	
6-8-1915	..	..	200	
7-8-1915	..	..	100	
8-8-1915	..	..	150	
9-8-1915	..	..	40	

The salient features of this case are :

- (1) Although the patient was sugar-free for nearly two months before death and the sugar concentration of the blood fairly normal during that period, yet the general health did not improve.
- (2) The wound and gangrenous condition of the feet healed very satisfactorily.
- (3) The carbohydrate tolerance was being regained despite the feeble state of the patient's general health.
- (4) No acidosis : no di-acetic acid.
- (5) Trypsogen appeared to have some slight controlling influence over the glycosuria.
- (6) The fatal termination was due to uræmic—not diabetic—coma.
- (7) The elimination of nitrogen in the urine early began to show signs of failure, and this was quickly followed by a decrease in the quantity of urine eliminated until there was virtual suppression. (*Vide Chart IV.*)

Diabetic No. X.—Hindu male, aged 42 years. Clerk. Before the onset of glycosuria—over three years ago—the patient was very stout and flabby. His eldest brother had diabetes, but the other members of his family were healthy.

He gave the usual history of polyuria, boils, abscesses, eczema of the leg. His diet was very highly carbonaceous and in addition he was very fond of sweets, consuming up to 2 lb. a day.

There was no history of diabetes in his father or mother or in their families.

The patient was admitted on the 12th June, 1915, and left hospital against advice on the 18th July, 1915, as soon as the eczema on his leg was cured. He regained some degree of carbohydrate tolerance but would not remain sufficiently long to be in a fit condition to return to his work and a non-restricted diet.

The result was that thirteen months afterwards he was re-admitted for gangrene of the foot and leg and eventually died from toxæmia and uræmia.

We have already quoted the complete figures for some days of this case on page 88. It will be sufficient now to give averages of the daily analyses for the different dietary periods.

Date.	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid, grms.	Acetone of urine, grms.	Glucose of blood, " "	Sugar of urine, grms.	Diet.	Grms.
13-6-1915 to 18-6-1915	2075	5.65	.608	.653	....	45.9	A { Milk { 1 st.* Vegets. { 6 oz. Lentils { 2 oz. Rice { 6 oz. Oatmeal { 4 oz. Soya Beans { 4 oz. Milk { 20 ½ st. Vegets. { 20 oz. Butter { 2 ½ oz. Eggs { 4 Oatmeal { 2 ½ oz. Fish { 4 z.	{ P=121.6 { C=130.1 { F=69.5
19-6-1915 to 26-6-1915	2540	6.14	.631	.661	.153	Trace one day	B { Do. plus Oatmeal { Fish {	{ P=56.8 { C=123.0 { F=102.4
27-6-1915 to 2-7-1915	2630	7.06	.375	.653	...	Nil	C { Do. plus Curds {	{ P=94.2 { C=123.0 { F=133.1
3-7-1915 to 9-7-1915	2610	7.05	.378	.641	.177	Nil	D { Do. plus Oatmeal { Potatoes {	{ P=97.4 { C=147.5 { F=134.7
One day - 10th - of relative carbohydrate starvation.								
10-7-1915	1650	3.55	.231	.415	....	Nil	E { Milk { ½ st. Vegets. { 20 oz. Butter { 2 oz. Milk { ½ st. Fish { 4 oz. Bread { 4 oz. Vegets. { 20 oz. Butter { 2 ½ oz. Eggs { 4 Curds { 8 oz. Potatoes { 3 oz.	{ P=30.6 { C=77.5 { F=83.5
11-7-1915 to 17-7-1915	2230	7.23	.57	Slight trace	.138	Nil	F { Milk { ½ st. Vegets. { 20 oz. Butter { 2 ½ oz. Eggs { 4 Curds { 8 oz. Potatoes { 3 oz.	{ P=96.4 { C=161.5 { F=140.5

\* 1 Seer (st.) = 32 oz.

The patient insisted on leaving hospital the next day so that the carbohydrate tolerance could not be raised to a higher level than a gross amount of 185 grammes.

This patient showed the same salient features as these previously treated by this method. There were no signs of acidosis and, once the sugar of the blood became normal, the eczema of the leg cleared up and the patient's general condition improved. His carbohydrate tolerance was increasing quite satisfactorily whilst he remained in hospital.

On the 15th August, 1916,—over a year later—the patient was readmitted. He gave a history of having remained free from sugar for over six months after leaving the hospital.

He now returns with gangrene of the foot, toxæmia, copious albuminuria, glycosuria and acetonuria. A slight injury to his foot began the trouble which rapidly developed into cellulitis and gangrene.

The man was very ill from the day of readmission—vomiting, restless, toxæmic and uræmic. No attempt was made to treat him as on the previous occasion. His diet was mainly milk, eggs and soup. Stimulants were given freely, but, although he improved for a fortnight or so and the wound began to heal, later the toxæmia increased, he became stuporous, the quantity of urine decreased, as in No. IX, and he sank into uræmic coma and died on the 14th September, 1916. There were never any signs of true diabetic coma. A short summary of the figures obtained may be given:—

Date.	Average daily quantity of urine. c.c.	Acetone of urine. grm.	Average of sugar of urine. %	Urea of blood. %	Di-acetic acid.
15-8-1916 to 31-8-1916	1210	Trace	·56	..	Nil.
1-9-1916 to 14-9-1916	815	Trace	·58	·08	Nil.

The last few days before death the excretion of urine fell to a few hundred c.c. daily.

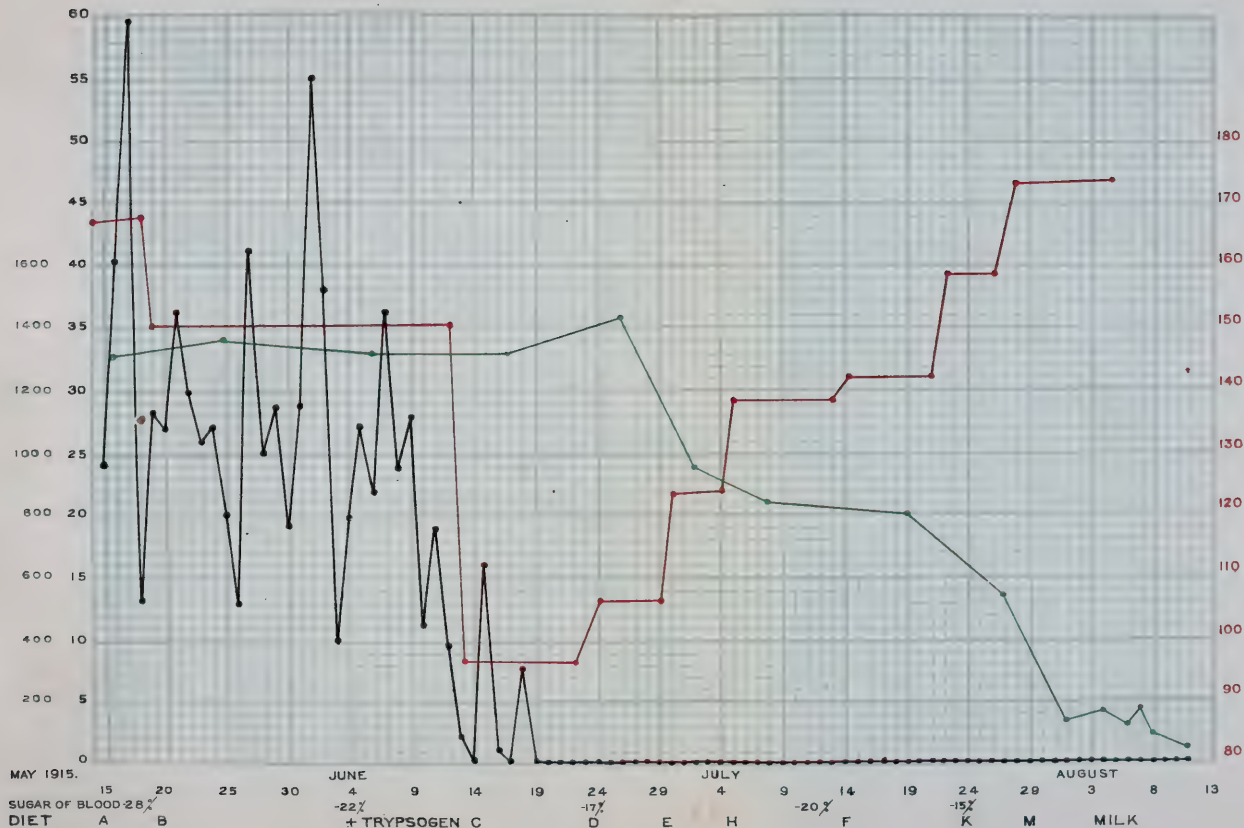
At this period of the enquiry we had commenced the chemical examination of the blood: this patient, however, was so seriously ill that we did not care to risk drawing off sufficient blood for complete



# CHART IV.

DIABETIC No. IX

DAILY RECORDS.



— Sugar of Urine, Grms.  
 — Carbohydrate Value of Diets, Grms.  
 — Average Quantity of Urine, C.C.

analysis. From the results obtained later in similar conditions there is little doubt but that his blood would have shown a very great retention of nitrogenous waste-products. This retention of non-protein nitrogen goes on increasing as the toxæmia and uræmia develop. This is a subject, however, to which we shall return when we come to deal with the cause of death in the ordinary mild types of diabetes met with in India.

Diabetic No. XVII.—Hindu male, aged 55 years. The patient was admitted for paralysis of right side which came on suddenly, he says, after a fright: a tree fell and was thought by him to have killed a man. There is no family history of diabetes, nor specific history. Lives on the ordinary Hindu type of diet.

This man was confined to bed and was much easier to watch and prevent from getting extra food than those able to move about. There was a history of polyuria for three months previous to admission. It is doubtful whether all the urine was saved during the period the patient was under observation.

This case was long in reacting to treatment and eventually the administration of an extract of liver appeared to influence the patient's condition beneficially.

The salient features of this case are :—

- (1) The low tolerance to carbohydrates on admission. On diet B, which contained only 55 grammes of carbohydrate, mostly from green vegetables, and about 30 grammes from protein metabolism, or 88 grammes in all the glycosuria continued.
- (2) On the same diet with the addition of liver extract, the sugar practically disappeared.
- (3) The hyperglycæmia was cleared by a few days of low dieting—diet C—the sugar of the blood being reduced from '26 per cent when the patient was on diet B to '17 per cent when on diet C.
- (4) The building-up of the patient's carbohydrate tolerance to a gross value of 140 grms. was fairly rapidly accomplished.



Date	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid, grms.	Acetone of urine, grms.	Glucose of blood, %	Sugar of urine, grms.	Grams.	Diet.
30-11-1915	900	..	..	Nil	..	49.5	Milk 12 oz.*	P- 44.5
1-12-1915	920	..	..	Nil	35	35.0	Vegets. 3	C- 191.0
2-12-1915	950	..	..	Nil	..	36.1	Eggs 2½ oz.	F- 90.2
3-12-1915	950	..	..	Nil	..	45.6	Butter 6 oz.	
							Rice	
The rice was replaced by washed curds.								
4-12-1915	1200	..	..	Nil	..	1.8		
5-12-1915	650	..	..	0.156	..	1.9		
6-12-1915	1400	..	..	0.28	..	4.2		
7-12-1915	900	..	..	0.17	..	Trace		
8-12-1915	1400	..	..	0.28	..	1.2		
9-12-1915	900	..	..	0.17	..	Trace		
10-12-1915	900	..	..	0.37	..	1.8	Milk 12 oz.	P- 113.1
11-12-1915	1300	..	..	0.72	..	3.9	Vegets. 6	C- 55.1
12-12-1915	600	..	..	0.45	..	2.4	Eggs 2½ oz.	F- 168.9
13-12-1915	1400	..	..	0.84	..	5.6	Butter 4	
14-12-1915	750	5.46	0.25	0.22	26	3.1	Curd 1	
The patient was unable to tolerate the carbohydrates of diet B.								
New extract of liver was administered thrice daily in drachm doses.								
15-12-1915	600	4.64	..	0.14	..	Nil		
16-12-1915	1300	12.93	0.42	0.87	..	Trace		
17-12-1915	800	3.76	0.32	0.15	..	Nil		
18-12-1915	900	5.23	0.18	0.18	..	2.7		
19-12-1915	900	9.74	0.49	0.31	..	1.4		
20-12-1915	700	7.64	0.30	0.42	..	Trace		
								Diet B plus liver extract.

\* 1 Seer (sr.) = 32 oz.

Date,	Quantity of urine, c.c.	Nitrogen of urine, grms.	Uric acid, grms.	Acetone of urine, grms.	Glucose of blood, %	Sugar of urine, grms.	Diet.	Grms.
21-12-1915	1500	11.01	.82	.024	..	Trace	{ Milk Vegts. 8 oz.* C-Eggs 3 Butter 2oz. Curds 1/4 sr }	{ P- 62.3 C- 43.9 F-129.7 }
22-12-1915	500	3.73	.20	Nil	..	do.		
23-12-1915	900	7.93	.56	Nil	..	do.		
Now the building-up process began.								
24-12-1915	1000	7.14	.37	Nil	.17	Trace.	{ Diet C plus Vegts : 8 oz. }	{ P- 66.8 C- 66.3 F-133.7 }
25-12-1915	350	2.08	.19	Nil	..	Nil		
26-12-1915	750	5.76	.25	Nil	..	Nil		
27-12-1915	800	6.03	.24	Nil	..	Nil		
28-12-1915	900	6.09	.27	Nil	..	Nil		
1 oz. of bread added.								
29-12-1915	600	4.90	.24	Nil	..	Nil	{ Do. plus Bread 1 oz. }	{ P- 69.3 C- 81.7 F-135.0 }
30-12-1915	950	9.74	.228	Trace	..	Nil		
31-12-1915	750	4.72	.225	Trace	..	Nil		
Another oz. of bread added.								
1-1-1916	800	5.75	.24	Nil	..	Nil	{ Diet C plus Vegts. 8 oz. }	{ P- 71.8 C- 97.1 F-136.3 }
2-1-1916	700	4.94	.28	Trace	165	Nil	{ Do. plus Bread 2 oz. }	
3-1-1916	700	3.52	.26	Trace	-	Nil		
Two oz. of potatoes added.								
4-1-1916	850	..	..	Nil	-	Nil	{ Do. plus Potatoes 2 oz. }	{ P- 73.2 C-108.2 F-137.0 }
5-1-1916	850	..	..	Nil	..	Nil		
6-1-1916	700	..	..	Nil	..	Nil		

The patient would not stay any longer in hospital, so that no further building-up was possible. However, his  $\alpha$  tolerance was increased by over 100 per cent by the treatment, and he left hospital very much improved in health.

\* 1 Seer (sr.) = 32 oz.

Diabetic No. XXIII.—Mrs. B., European female, aged 59. She is the mother of 11 children of which four are living. No family history of diabetes.

After the climateric the patient became very stout and ten years ago sugar was discovered in her urine. She was dieted and lost weight and the sugar disappeared. Her weight before the first attack of glycosuria was 240 lb.

Two years ago, after a gradual increase in weight up to nearly the former figure, glycosuria recurred and again her weight began to fall. She came to hospital passing abundant sugar and weighing 159 lb. During the greater part of the eight years intervening between the first and second attacks the patient was quite fit and healthy on a slightly restricted carbohydrate diet.

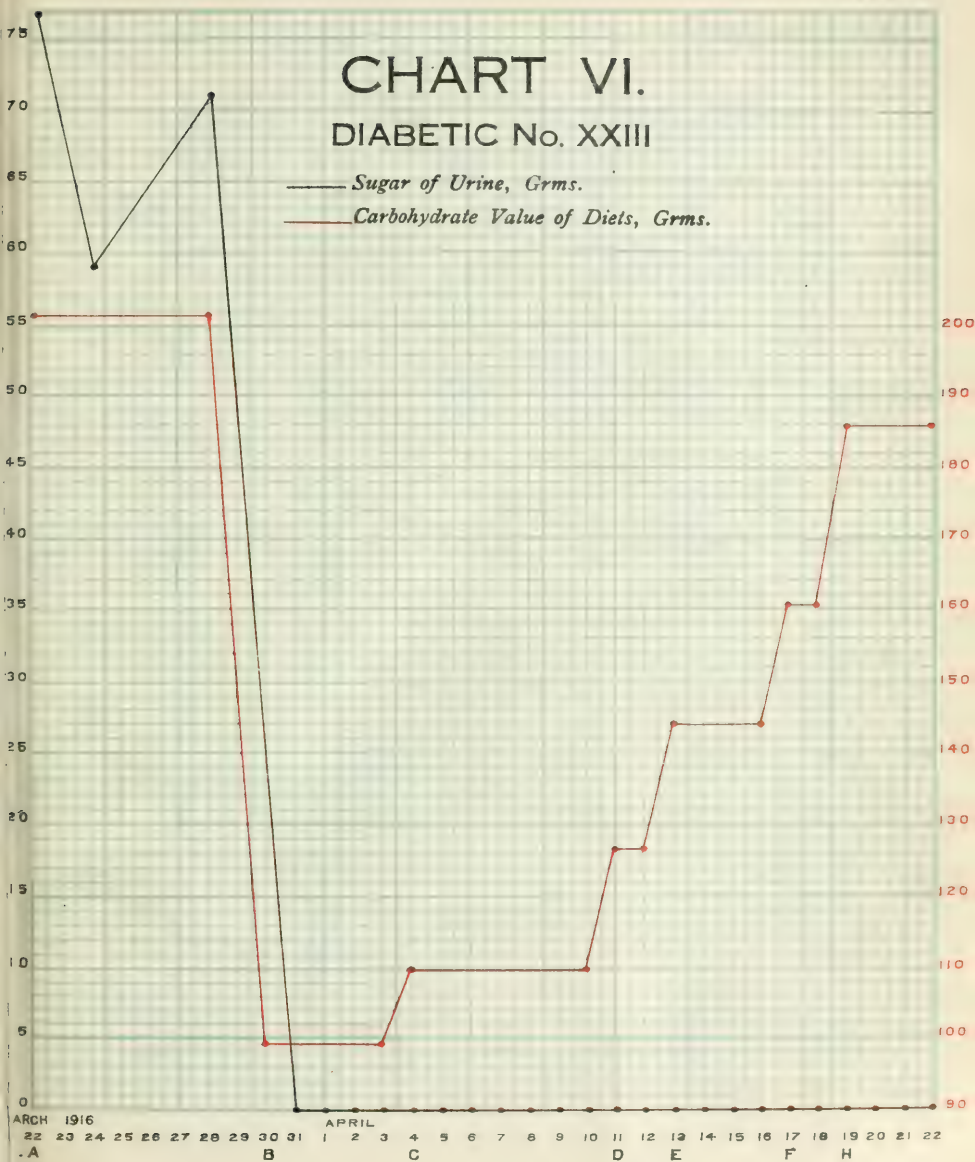
The treatment of this type of case is not difficult so long as the patient will obey loyally the directions given.

Date.	Quantity of urine cc.	Sugar of urine, grms.	Diet	Grms.
22-3-1916 to 28-3-1916	1550	0.6	A { Milk 1 sl. Vegts. 8 oz. Bread 8 oz. Butter 2 oz. Fish 4 oz. }	P=72 C=202 F=108
31-3-1916 to 3-4-1916	1170	Nd	B { Milk 1 sl. Vegts. 20 oz. Butter 2 oz. Fish 1 oz. }	P=58.2 C=399.0 F=106.5
4-4-1916 to 10-4-1916	950	Nd	C { Diet B plus Potatoes 2 oz. }	P=59.5 C=419.0 F=107.2
11-4-1916 to 12-4-1916	950	Nd	D { Do. plus Bread 1 oz. }	P=63 C=427 F=108
13-4-1916 to 16-4-1916	870	Nd	E { Do. plus Bread 1 oz. }	P=65.5 C=444.0 F=109.0
17-4-1916 to 18-4-1916	1100	Nd	F { Do. plus Bread 1 oz. }	P=68.0 C=461.0 F=110.2
19-4-1916 to 22-4-1916	900	Nd	H { Do. plus Bread 1 oz. }	P=70.5 C=487.6 F=111.4

# CHART VI.

DIABETIC No. XXIII

— *Sugar of Urine, Grms.*  
— *Carbohydrate Value of Diets, Grms.*



supplied to them. We have been accustomed in this class of patient to demonstrate a simple Fehling test for sugar, so that the patient is able to carry out his own dietary experiments in an intelligent manner.

At the same time we supply those educated with a short list—similar to that on page 147 which gives the carbohydrate value in grammes per ounce of the different food-material in common use in India. By means of this list any intelligent patient is able to maintain a dietary of a certain carbohydrate value and yet vary the constituents from day to day. He is also able to increase the starch of his dietary gradually and know exactly by how much he has done so.

We have had large numbers of patients in all parts of India carrying on their own treatment in this manner. Most of them are in places where skilled medical advice is impossible to obtain: however, even when the patient happens to live where medical attendance is possible, this method of testing his own urine increases the interest in his condition and acts as an incentive to persevere with the necessary dietary restrictions. So far as our experience goes, there is practically no danger of diabetic coma in India from a too drastic cutting down of the carbohydrates; and, of course, in all cases where we permit the above routine the patient has been under our observation and treatment and has been considered a safe case to be allowed to carry on with his own treatment.

Diabetic No. XXX.—Hindu male, aged 28 years. Farmer. He gave the usual history and signs. Glycosuria was discovered a year ago. He lived on rice, lentils, milk and vegetables to a large extent. During the last months he lost heavily in weight and on admission only weighed 70 lb. There was no family history of diabetes.

He was admitted on the 21st June, 1916, and left hospital on the 19th August, 1916.

Date.	Quantity of urine. c.c.	Acetone of urine.	Sugar of blood. %	Sugar of urine. grms.	Diet.	Grms.
21-6-1916 ..	800 †	Nil	..	24.0	A { Milk 1 sr. Vegts. 20 oz. Butter 2½ oz. Eggs 4 }	P—58.0
22-6-1916 ..	1100	Nil	.28	22.0		C—99.0
23-6-1916 ..	1350	Nil	..	27.0		F—126.0

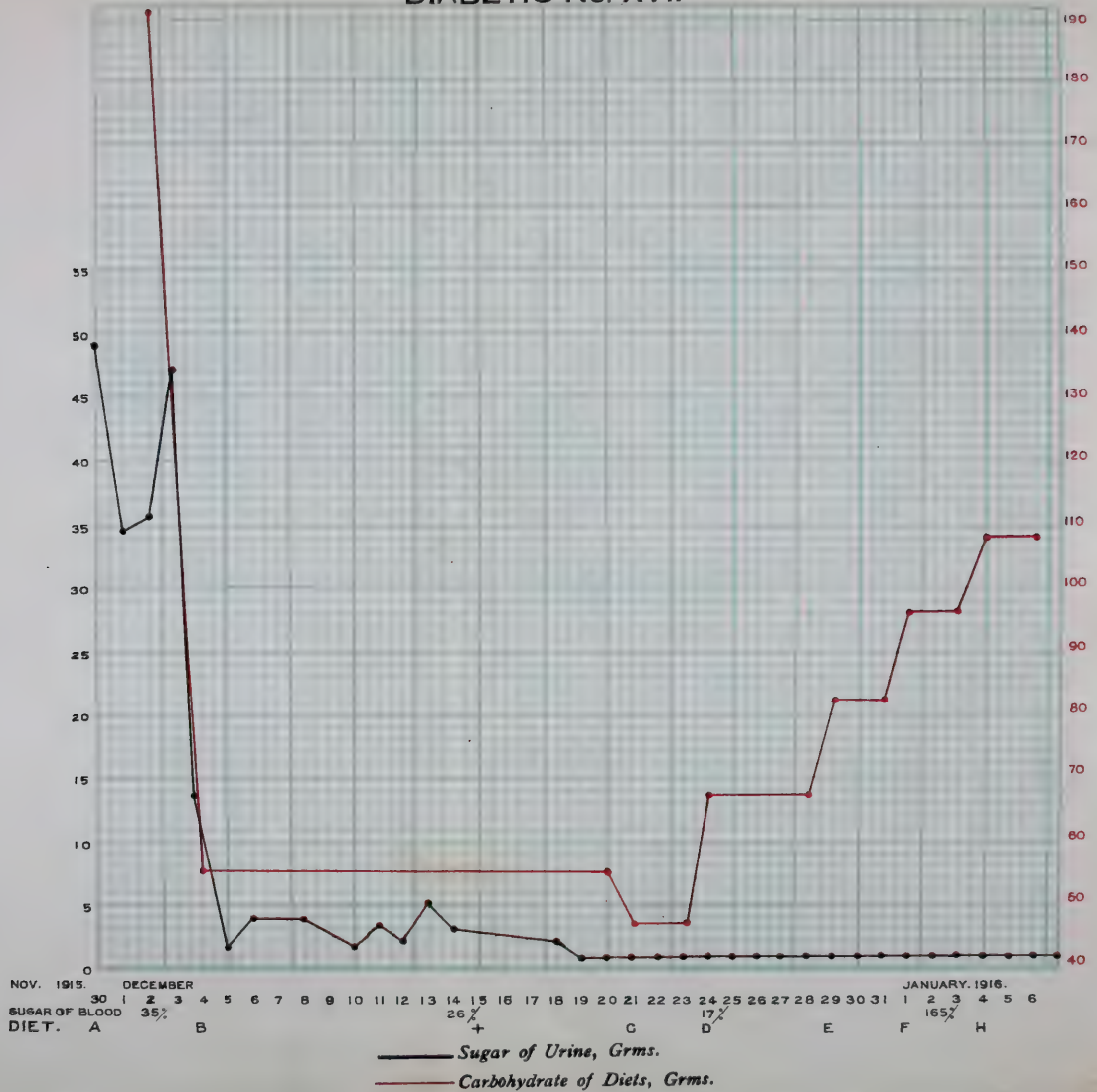
Next day the patient stole some bread which put up the excretion of sugar nearly four-fold.





# CHART V.

## DIABETIC No. XVII





Date.	Quantity of urine. c.c.	Acetone of urine.	Sugar of blood. %	Sugar of urine. grms.	Diet.	Grms.
26-6-1916 ..	850	<i>Nil</i>	..	<i>Nil</i>	B { Milk 1 sr. Vegts. 12 oz. Butter 2½ oz. Eggs 2 }	P— 49.5
27-6-1916 ..	1700	<i>Nil</i>	..	<i>Nil</i>		C— 76.6
28-6-1916 ..	?	<i>Nil</i>	.07	<i>Nil</i>		F—115.0
29-6-1916 ..	1700	<i>Nil</i>	..	<i>Nil</i>		
30-6-1916 ..	1200	<i>Nil</i>	..	<i>Nil</i>	} Diet A	
1-7-1916 ..	1200	<i>Nil</i>	..	<i>Nil</i>		
Diet slightly increased.						
2-7-1916 ..	1000	<i>Nil</i>	..	<i>Nil</i>	C { Diet A + Eggs 2 Potatoes 2 oz.	P— 63.2
3-7-1916 ..	1400	<i>Nil</i>	.107	<i>Nil</i>		C—110.0
4-7-1916 ..	1600	<i>Nil</i>	..	<i>Nil</i>		F—131.6
5-7-1916 ..	1400	<i>Nil</i>	..	<i>Nil</i>		
Another slight increase.						
6-7-1916 } to 10-7-1916 }	1320	<i>Nil</i>	..	<i>Nil</i>	D { Milk ¾ sr. Vegts. 28 oz. Butter 2½ oz. Eggs 6 Potatoes 3 oz.	P— 58.1 C—127.5 F—122.5
The protein and fat increased.						
11-7-1916 } to 15-7-1916 }	1100	<i>Nil</i>	.14	<i>Nil</i>	E { Diet C + Curds 4 oz.	P— 76.8 C—127.5 F—137.8
Diet slightly increased.						
16-7-1916 } to 20-7-1916 }	1160	<i>Nil</i>	..	<i>Nil</i>	F { Diet C + Milk ¼ sr. + curds 4 oz.	P— 86.5 C—138.8 F—148.8
Protein increased.						
21-7-1916 } to 28-7-1916 }	1420	<i>Nil</i>	.128	<i>Nil</i>	G { Do + Fish 4 oz.	P— 94.9 C—138.2 F—150.0
Protein decreased, carbohydrate increased.						
29-7-1916 } to 6-8-1916 }	1700	<i>Nil</i>	.127	<i>Nil</i>	H { Milk ¾ sr. Vegts. 28 oz. Butter 2½ oz. Eggs 6 Potatoes 4 oz. Curds 4 oz. Bread 1 oz.	P— 74.3 C—149.7 F—140.8
Carbohydrate increased.						
7-8-1916 } to 14-8-1916 }	2120	<i>Nil</i>	..	<i>Nil</i>	K { Milk 1 sr. Vegts. 20 oz. Butter. 2½ oz. Eggs 6 Potatoes 6 oz. Fish 4 oz. Bread 2 oz.	P— 79.6 C—165.2 F—134.6
All constituents slightly increased.						
15-8-1916 } to 19-8-1916 }	1860	<i>Nil</i>	.123	<i>Nil</i>	M { Diet E + Fish 4 oz. Bread 1 oz.	P— 88.5 C—182.2 F—137.8

During the two months in hospital the patient gained 9½ lb. in weight and his general health had much improved.

After the lapse of the first few days this patient's conduct was exemplary. He eventually was discharged from hospital with a very much increased carbohydrate tolerance. On admission he failed to tolerate 100 grammes of carbohydrate of diet A, whereas when he left the diet was quite sufficient for all bodily requirements and he could tolerate 180 grammes of carbohydrates.

We did not expect this patient to improve to the extent the record shows. He was very thin and emaciated with very little reserve to fall back on. One noteworthy point is that even on a very poor carbohydrate diet—diet B—he did not pass even a trace of acetone in his urine.

Diabetic No. XXXI.—This is a very similar type of case to No. XXX.—Hindu male, aged 40. Glycosuria for about six months following on an attack of fever. The patient has lived on ordinary Bengali diet—largely carbonaceous.

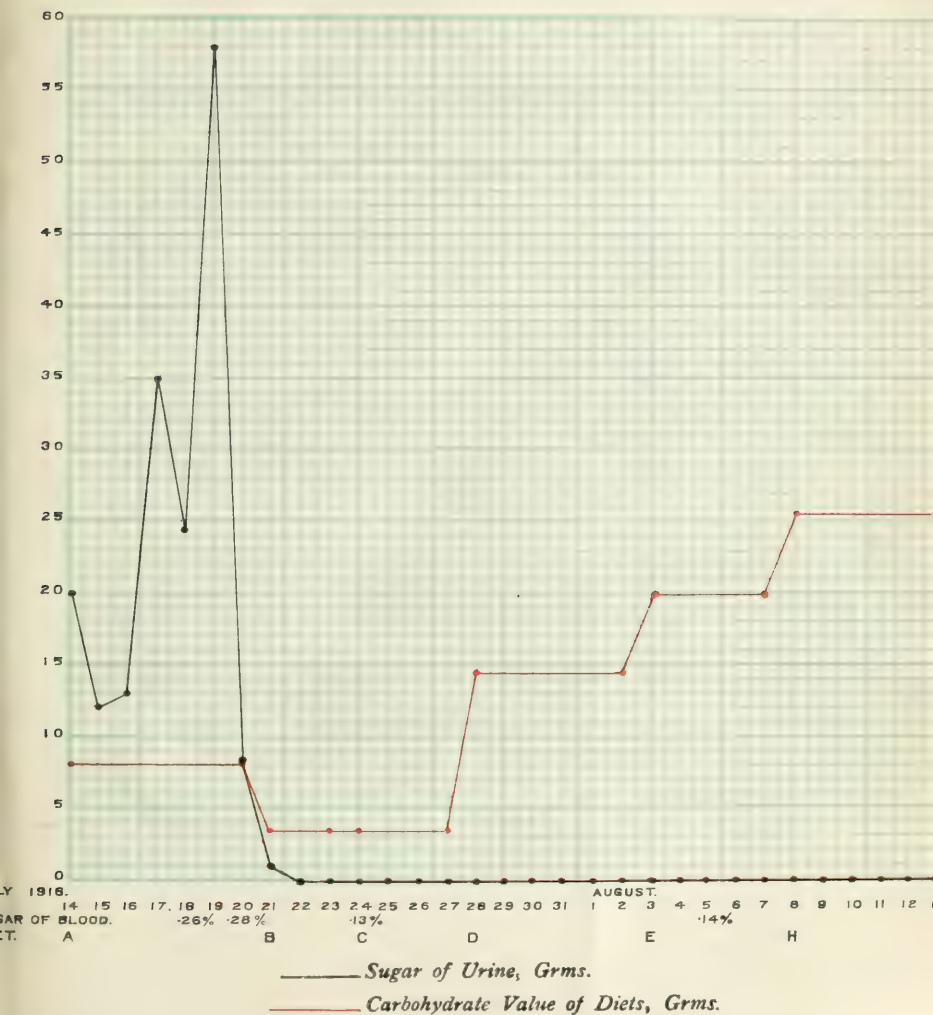
Weight on admission, 99 lb. He had lost a considerable amount of weight since the polyuria set in.

He was admitted on the 11th July, 1916, and died on the 14th August, 1916. Death was due to pneumonia and pericarditis.

Date.	Quantity of urine, c.c.	Acetone of urine, grms.	Glucose of blood, %	Sugar of urine, grms.	Diet.	Grms.
14-7-1916 } to 20-7-1916 }	1100	Present { 26 28 }		25.0	A { Milk 4 sr. Vegts. 16 oz Butter 2½ oz Eggs 4 }	{ P—55.7 C—87.8 F—122.0
The diet was further decreased.						
21-7-1916 ..	1160	Present ..	88		B { Milk 4 sr. Vegts. 20 oz. Butter 2½ oz.	P—30.6
22-7-1916 ..	550	Nil	..	Trace		C—77.5
23-7-1916 ..	900	Nil	..	Nil		F—95.5
Protein increased.						
24-7-1916 } to 27-7-1916 }	1250	Trace	43	Nil	C { Do. plus Eggs 4 }	{ P—38.6 C—77.5 F—105.5
Carbohydrate increased.						
28-7-1916 } to 2-8-1916 }	2150	0.54	..	Nil	D { Do. plus Vegts. 8 oz.	{ P—43.0 C—99.9 F—109.5
Diet increased in all respects.						
3-8-1916 ..	3100	0.69	..	Nil	E { Milk ½ sr. Vegts. 28 oz Butter 2½ oz. Eggs 6 Curds 4 oz.	{ P—75.4 C—110.4 F—138.0
4-8-1916 ..	3100	0.69	..	Nil		
5-8-1916 ..	2600	0.48	14	Nil		
6-8-1916 ..	1700	0.38	..	Nil		
7-8-1916 ..	3600	0.56	..	Nil		
Carbohydrate increased.						
8-8-1916 ..	3300	0.528	..	Nil	H { Do. plus Potatoes 2 oz.	{ P—76.6 C—121.5 F—138.8
9-8-1916 ..	2000	Nil	..	Nil		
10-8-1916 ..	2000	Nil	..	Nil		

# CHART VIII.

DIABETIC No. XXXI

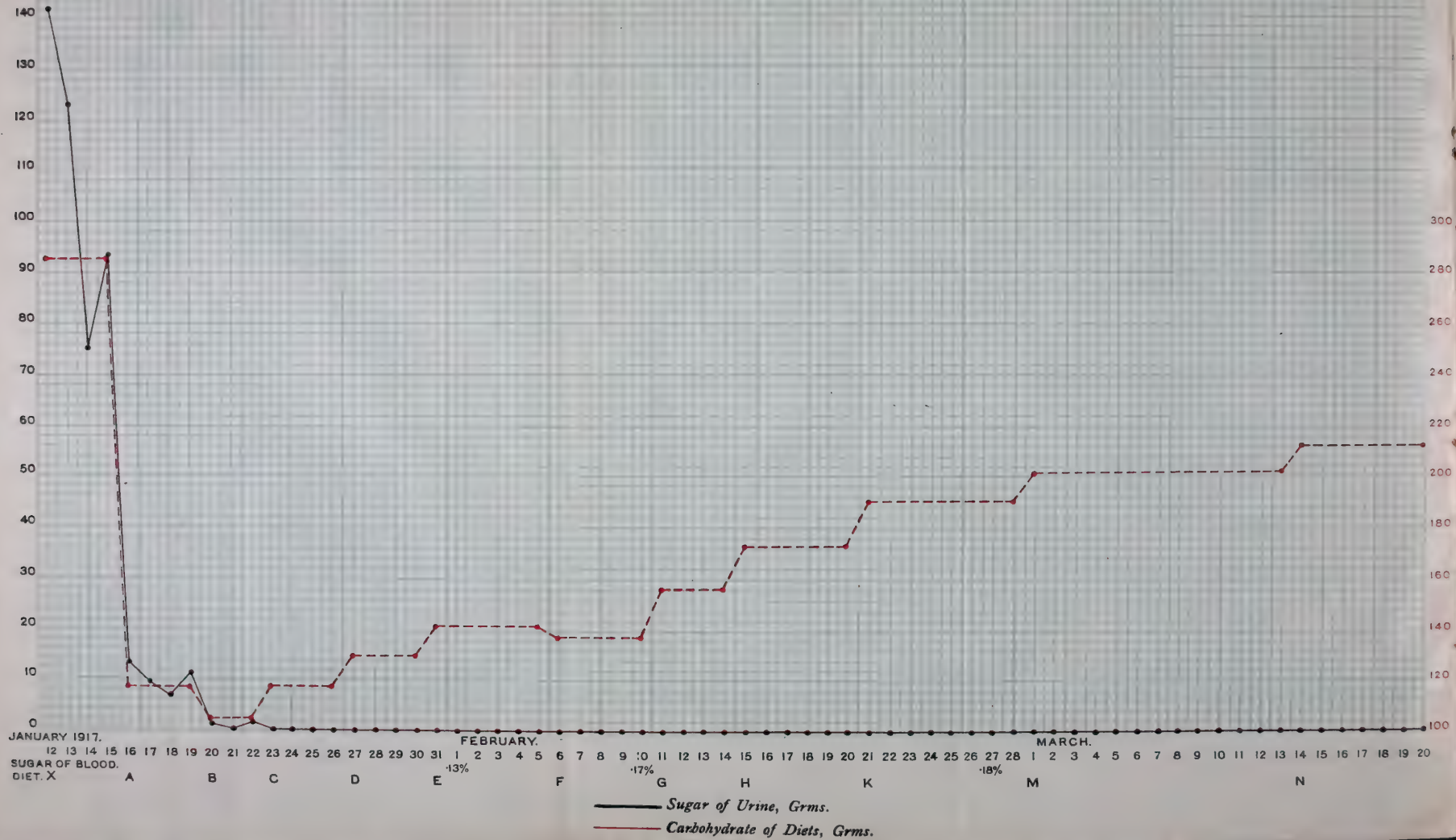






# CHART IX.

## DIABETIC No. XLV







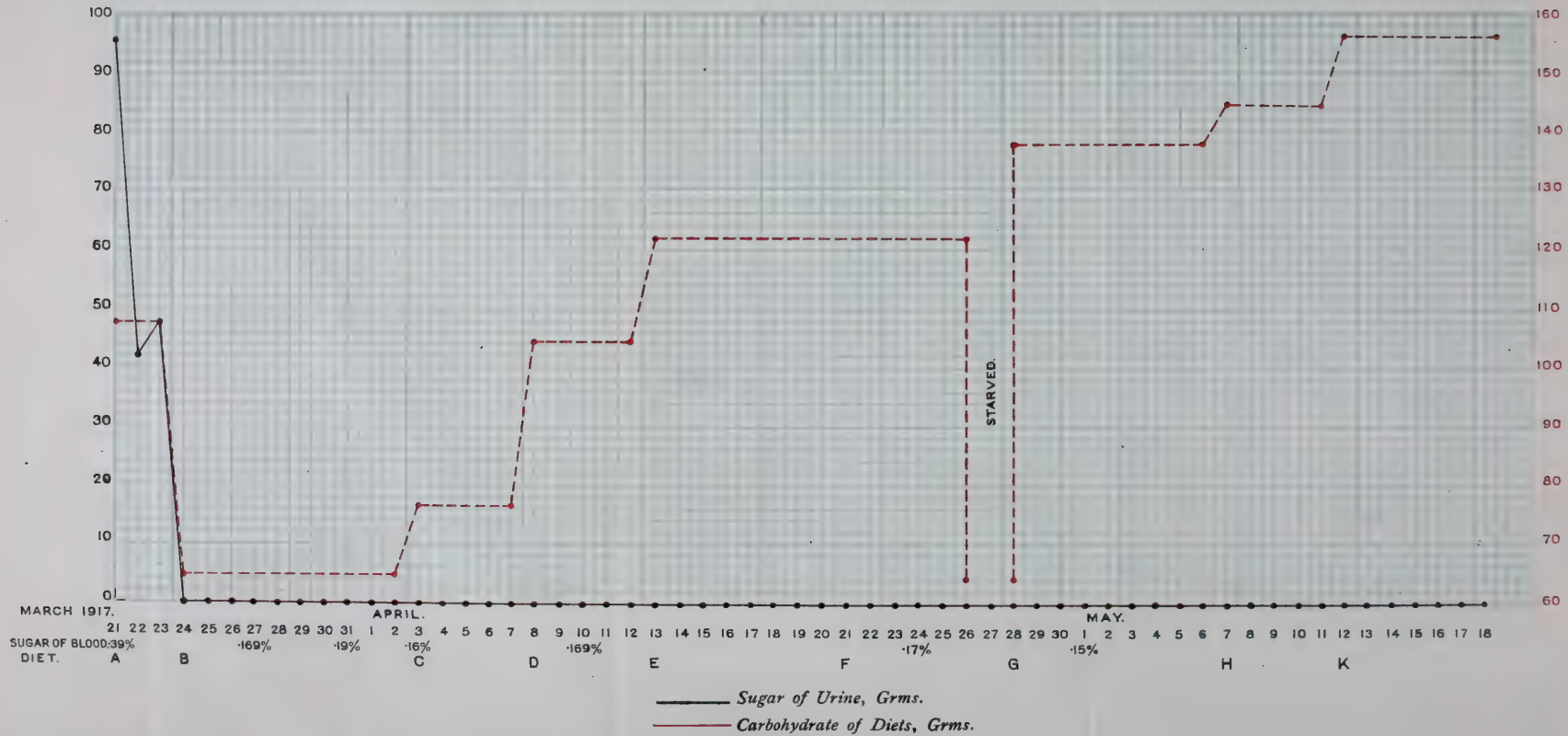
Date.	Quantity of urine. c.c.	Acetone.	Glucose of blood. %	Sugar of urine. grms.	Diet.	Grms.
23-1-1917 to 26-1-1917 } ..	1270	Nil	..	Nil	C { Diet A Eggs 2	{ P— 60.8 C—118.6 F—116.1
A further carbohydrate increased.						
27-1-1917 to 30-1-1917 } ..	1600	Nil	..	Nil	D { Do. Vegts. 4 oz.	{ P— 62.3 C—129.8 F—118.1
A still further carbohydrate increased.						
31-1-1917 to 5-2-1917 } ..	1630	Nil	13	Nil	E { Milk 1 sf. Vegts. 20 oz. Bread 2 oz. Eggs 4 Potatoes 2 oz.	{ P— 64.2 C—140.8 F—118.8
Protein and fat decreased.						
6-2-1917 to 10-2-1917 } ..	1850	Nil	17	Nil	F { Milk 3 sf. Vegts. 16 oz. Eggs 4 Butter 2 oz. Bread 3 oz. Potatoes 2 oz.	{ P— 54.7 C—139.2 F—106.1
Carbohydrate increased.						
11-2-1917 to 14-2-1917 } ..	1475	Nil	18.7	Nil	G { Diet Do. plus Bread 1 oz.	{ P— 57.3 C—156.2 F—107.1
Greater carbohydrate increased.						
15-2-1917 to 20-2-1917 } ..	900	Nil	..	Nil	H { Do. Bread 1 oz.	{ P— 59.8 C—173.2 F—108.0
Still greater carbohydrate increased.						
21-2-1917 to 28-2-1917 } ..	1140	Nil	18	Nil	K { Do. Bread 1 oz.	{ P— 62.3 C—190.2 F—109.0
1-3-1917 to 13-3-1917 } ..	1150	Nil	18.7	Nil	M { Milk 1 sf. Vegts. 12 oz. Eggs 4 Butter 1½ oz. Bread 6 oz. Potatoes 4 oz. Cutlets 2	{ P— 90 C—201 F—110
All constituents increased.						
14-3-1917 to 20-3-1917 } ..	about 1200 daily	Nil	..	Nil	N { Milk 1 sf. Vegts. 16 oz. Eggs 4 Bread 6 oz. Potatoes 4 oz. Butter 2 oz.	{ P— 90 C—212 F—120
Fish or chicken or mutton 6 oz. daily. The patient left weighing 160 lb.						

This patient improved most satisfactorily and returned home quite satisfied with the type and quantity of the dietary on which she had lived during the last month of her stay in hospital. She wrote some



# CHART X.

DIABETIC No 169



months afterwards to inform us that she was keeping to the dietetic restrictions and was remaining sugar-free.

As will be evident from the synopsis of the daily analyses, she was discharged with the level of her carbohydrate tolerance raised by over 100 grammes; this permitted of a dietary quite sufficient for all physiological requirements.

Never at any time during the course of the treatment did the urine show any acetone or diacetic acid.

Diabetic No. 169.—Bengali: aged 51. He gives a history of polyuria and glycosuria for four years. Suffered from renal colic in 1903. Accustomed to a dietary very rich in carbohydrates and sugar. After 1912 he became very fat.

Admitted 21st March, 1917, with very high glycosuria, and albuminuria.

The knee-jerks were lost: he complained of tingling and numbness of the feet and legs and the patient had all the appearances of early uræmia.

He looked ill, slightly oedematous, the urine very scanty in quantity for the percentage of sugar present, and the chemical examination of the blood showed severe retention of nitrogenous waste-products—the urea of the blood on admission was 0.1 per cent and the relationship of

$$\frac{\text{Non-protein nitrogen}}{\text{Total nitrogen}} = \frac{0.07}{3.22} = \frac{1}{46}$$

which is about three times the normal figure. In addition to the treatment of the glycosuria, this man was purged, given hot air-baths, etc., to prevent the onset of uræmic coma. He improved rapidly under treatment and left hospital on the 18th May, 1917, free from albumen and sugar and able to tolerate a fairly substantial dietary.

This was a most interesting case from many points of view, and exhibits the effects of treatment exceedingly well.

The building-up of the carbohydrate tolerance was accomplished in the usual manner. It was increased from about 70 to over 150 grammes per day. The method adopted was to decrease the carbohydrate intake below the patient's tolerance—diet A was too high so diet B was tried—and then slowly build up all constituents of the diet. In some periods we increased the protein and fat—as in diet F—in other periods the carbohydrate element alone was increased.

By cautious dieting in conjunction with daily examinations of the urine and occasional examinations of the blood the patient's tissues can be re-educated to utilize fairly large quantities of starches.

Date.	Quantity of urine, c.c.	Acetone of urine, grms.	Glucose of blood, %	Urea of blood, %	T.N. of blood, %	N.P.N. of blood, %	Sugar of urine, grms.	N.P.N. T.N.	Diet.	Grms.
21-3-1917	1200	N/d	39	10	3.22	97	96	1.46	{ Milk Rice } A	P—29 C—108.5
22-3-1917	500	N/d	..	..	..	..	42	..	{ Sugar Soup } A	P—187 C—187
23-3-1917	800	..	..	..	..	..	48	..	..	..
Semi-starvation.										
24-3-1917	700	N/d	..	..	..	..	N/d	..	{ Milk Vegets. } B	P—398 C—678
25-3-1917	600	N/d	..	..	..	..	N/d	..	{ Butter Eggs } B	P—810 C—810
26-3-1917	500	N/d	..	..	..	..	N/d	..	..	..
27-3-1917	1100	N/d	469	06	3.04	956	N/d	1.54	..	..
28-3-1917	500	0.85	..	..	..	..	N/d	..	..	..
29-3-1917	450	0.17	..	..	..	..	N/d	..	..	..
30-3-1917	600	N/d	19	08	3.38	97	Trace	1.48	Barley water one day.	..
31-3-1917	1250	N/d	..	..	..	..	N/d	..	..	..
1-4-1917	1000	N/d	..	..	..	..	N/d	..	..	..
2-4-1917	1600	N/d	..	..	..	..	N/d	..	..	..
Diet slightly increased										
3-4-1917	2250	N/d	16	04	..	..	N/d	..	{ Milk Vegets. } C	P—36.6 C—77.5
4-4-1917	1850	N/d	..	..	..	..	N/d	..	{ Butter Eggs } C	P—77.5 C—77.5
5-4-1917	2200	N/d	..	..	..	..	N/d	..	..	..
6-4-1917	1500	N/d	..	..	..	..	N/d	..	..	..
7-4-1917	1850	N/d	..	..	..	..	N/d	..	..	..
A further increase, all round.										
8-4-1917	1850	N/d	..	..	..	..	N/d	..	{ Milk Vegets. } D	P—30.8 C—105.2
9-4-1917	1650	N/d	..	..	..	..	N/d	..	{ Butter Eggs } D	P—91.8 C—91.8
10-4-1917	1350	N/d	169	06	3.65	938	N/d	1.08	..	..
11-4-1917	1000	N/d	..	..	..	..	N/d	..	..	..
12-4-1917	850	N/d	..	..	..	..	N/d	..	{ Bread } D	..

T.N.—Total nitrogen N.P.N.—Non-protein nitrogen.

Date.	Quantity of urine, c.c.	Acetone, grms.	Glucose of blood, %	Urea of blood, %	T.N. of blood, %	N.P.N. of blood, %	Sugar of urine, grms.	N.P.N. T.N.	Diet.	Grms.
13-4-1917	1300	Nd	Carbohydrates increased.				Nd	..	E Diet D plus 1 oz. Bread 1 oz.	P-53.3 C-122.2 F-93.0
14-4-1917	1400	Nd	..	..	..	..	Nd	..		
15-4-1917	1400	Nd	..	..	..	..	Nd	..		
16-4-1917	1600	Nd	..	..	..	..	Nd	..		
17-4-1917	1000	Nd	..	..	..	..	Nd	..		
18-4-1917	3000	Nd	..	..	..	..	Nd	..		
19-4-1917	2800	Nd	..	..	..	..	Nd	..		
20-4-1917	2200	Nd	..	..	..	..	Nd	..		
Protein and fat increased.										
21-4-1917	2200	Nd	..	..	..	..	Nd	..	F Milk 3 st. oz. Vegts. 20 oz. Butter 2 1/2 oz. Eggs 4 oz. Bread 2 oz. Fish 8 oz.	P-70.2 C-122.2 F-116.7
22-4-1917	3200	Nd	..	..	..	..	Nd	..		
23-4-1917	1400	Nd	..	..	..	..	Nd	..		
24-4-1917	2300	Nd	47	96	..	..	Nd	..		
25-4-1917	2800	Nd	..	..	..	..	Nd	..		
26-4-1917	1800	Nd	..	..	..	..	Nd	..		
One day—starvation.										
27-4-1917	3000	Nd	..	..	..	..	Nd	..	No diet.	
Carbohydrates and fats increased.										
28-4-1917	2450	Nd	..	..	..	..	Nd	..	G Milk 3 st. oz. Vegts. 16 oz. Butter 2 1/2 oz. Eggs 4 oz. Fish 8 oz. Bread 3 oz. Oil 1 oz.	P-70.3 C-128.2 F-129.3
29-4-1917	2100	Nd	..	..	..	..	Nd	..		
30-4-1917	1750	Nd	..	..	..	..	Nd	..		
1-5-1917	2000	Nd	45	96	3.68	0.49	Nd	1.75		
2-5-1917	3300	Nd	..	..	..	..	Nd	..		
3-5-1917	3350	Nd	..	..	..	..	Nd	..		
4-5-1917	2100	Nd	..	..	..	..	Nd	..		
5-5-1917	2200	Nd	..	..	..	..	Nd	..		
6-5-1917	3350	Nd	..	..	..	..	Nd	..		

T.N. Total nitrogen.

N.P.N. Non-protein nitrogen.

Date.	Quantity of urine, c.c.	Acetone, grms.	Glucose of blood, %	Urea of blood, %	T.N. of blood, %	N.P.N. of blood, %	Sugar of urine, grms.	N.P.N. T.N.	Diet.	Grms.
7-5-1917	2400	N/l	..	..	Carbohydrates increased.				Diet F plus Bread 1 oz. H	P- 72.5 C-145.2 F- 130.1
8-5-1917	3000	N/l	..	..	..	..	N/l	..		
9-5-1917	3000	N/l	..	..	..	..	N/l	..		
10-5-1917	2000	N/l	..	..	..	..	N/l	..		
11-5-1917	1600	N/l	..	..	..	..	N/l	..		
12-5-1917	3300	N/l	..	..	Carbohydrates further increased.				Do. plus Potatoes 2 oz. K	P- 73.7 C-156.3 F- 130.8
13-5-1917	2800	N/l	..	..	..	..	N/l	..		
14-5-1917	2200	N/l	..	..	..	..	N/l	..		
15-5-1917	1900	N/l	..	..	..	..	N/l	..		
16-5-1917	1700	N/l	..	..	..	..	N/l	..		
17-5-1917	3100	N/l	..	..	..	..	N/l	..		
18-5-1917	2300	N/l	..	..	..	..	N/l	..		

T.N. — Total nitrogen.      N.P.N. — Non-protein nitrogen.



This, however, was only one part of the problem presented by this patient. The other and more urgent condition requiring treatment was the state of the urinary excretion.

He came to us giving history of scanty urine with a very high percentage of sugar—13 per cent according to his statement. He showed 8 per cent of sugar during the first two days in hospital on a diet containing just over 100 grammes of carbohydrates. For thirteen days after admission the flow of urine was poor, and his general condition gave rise to considerable anxiety. After that period the urine began to be excreted freely and the patient showed marked signs of improvement.

This was reflected in the figures obtained from chemical examination of the blood. In 1916 we began to estimate the urea of the blood in addition to the sugar, and, in serious cases, we also determined the percentage of non-protein nitrogen. In 1917 we took up this line of research extensively and have now records of hundreds of analyses of the blood of patients' suffering from diabetes, kidney disease, failing heart, jaundice and other conditions.

Our figures for normal individuals show that the relationship of the non-protein nitrogen to the total nitrogen of the blood should be as 1 is to 120 or more. Any figure below 100 may be looked on as the beginning of the retention of nitrogenous waste-products. Where uræmia is present or inevitable the relationship may fall to 1 : 10 or lower. In one case of diabetes, dying in coma, the relationship  $\frac{\text{non-protein nitrogen}}{\text{total nitrogen}}$  was  $\frac{1}{5.53}$ . Before we began the analyses of the blood in diabetics dying in coma, we were inclined to accept the ordinary diagnosis that the coma was diabetic. Now we regard the great majority of these cases as examples of uræmic coma.

With this slight but necessary digression we may return to the patient under discussion.

When admitted his blood showed 0.1 per cent of urea and a ratio of  $\frac{\text{N.P.N.}}{\text{T.N.}} = \frac{1}{46}$ . He was passing a small amount of urine, albumen was present and there was some œdema. No great improvement was shown by the blood, except the disappearance of the hyperglycæmia, until a free flow of urine had become established; then analysis of the blood on the 10th April, 1917, gave a ratio of  $\frac{\text{N.P.N.}}{\text{T.N.}} = \frac{1}{108}$ , a fairly normal condition.

The fear of uremia had now been largely dispelled and we felt justified in increasing all constituents of the diet, including protein.

The blood was taken twice afterwards for analysis, but the first samples for T.N. and N.P.N. estimations were broken during distillation: on the second occasion the relationship was  $\frac{1}{75}$ . The patient, however, was passing plenty of urine so that no anxiety was felt as to his condition.

Three and a half months afterwards the patient came to show himself and we seized the opportunity to carry out another examination.

He stated that he was having a fairly liberal diet which included some rice and bread. The analyses showed:—

Date	T.N. of blood. %	N.P.N. of blood. %	Urea of blood. %	Glucose of blood. %	Fat of blood. %	Alkalinity of blood.	Sugar of urine %	Albumen of urine.	Acetone of urine.
16.8.1917	3.63	.084	.04	22	.08	$\frac{n}{32.5}$	.08	Nd	Nd
<div style="display: flex; justify-content: center; align-items: center;"> <div style="margin-right: 20px;">The ratio</div> <div style="display: flex; flex-direction: column; align-items: center;"> <div>N.P.N. .084</div> <div>T.N. 3.63</div> <div>1</div> <div>43</div> </div> </div>									

He had, therefore, fallen back more or less to the state he was in when first admitted to hospital.

This case illustrates very clearly the great change that can be effected in the chemical composition of the blood in the early stages of uræmia by dieting. Whether purgatives and diaphoretics are of very much assistance we gravely doubt.

This is a subject, however, to which we shall return, when dealing with the work done on the chemical examination of the blood in different diseases.

Diabetic No. CCLXX. Mrs. C., Polish. History of sugar for two years. Admitted in a low condition with three large carbuncles on her back and buttocks. There was albumen in the urine but no casts. The quantity of urine was small.

The glycosuria was slight but there was considerable hyperglycæmia on admission.

This patient's glycosuria was treated in the usual way and after she began to improve in general health the carbohydrate tolerance was built up to a moderate extent.

We are, however, giving the results of the analyses of the blood and urine in the case to demonstrate the chemical changes that can be effected in the blood and urine by dieting. At the same time we wish to show that in accordance with the improvement in the condition of the blood the patient's general health gradually returned to a fairly normal state.

This patient's serious condition was due very much more to the deficiency in renal excretion than to the diabetes.

The blood sugar on the day of admission was 0.3 per cent, yet the percentage of sugar in the urine was very low—practically only a trace. Under ordinary conditions of kidney excretion the urine should have shown between 3 and 4 per cent. Further, the ratio  $\frac{\text{N.P.N.}}{\text{T.N.}} = \frac{1}{29}$  points to very severe retention of nitrogenous waste-products. This retention lasted quite a long time and during that period the patient showed no signs of improvement—the albuminuria persisted and, despite a low carbohydrate diet, the sugar of the blood remained high. As soon as improvement set in, the nitrogenous waste retention decreased rapidly, the blood sugar came down to normal, albuminuria and glycosuria disappeared and the signs of uræmia and toxæmia cleared up; at the same time the carbuncles cleaned and became healthy healing ulcers.

*The Treatment of Diabetes in India.*

Date.	T.N. of blood. %	N.P.N. of blood. %	Urea of blood. %	Glucose of blood. %	Fat of blood. %	N.P.N. T.N.	Sugar of urine. %	Albumen of urine.	Acetone of urine.	REMARKS.
7-5-1917	3.34	.116	.10	.30	.16	1 <sup>st</sup>	.3	+	+	Conditions serious, toxic- mic and uræmia feared.
8-5-1917 to	..	..	..	..	..	..	Trace	+	+	Patient not doing well; gradually losing ground.
13-5-1917	..	..	..	..	..	..	Trace	+	+	Diet: 70 grms. Protein 100 " Carbo- hydrate.
14-5-1917 to	..	..	..	..	..	..	Trace	+	+	Diet 50 grms. Protein 77 " Carbo- hydrate.
18-5-1917	..	..	..	..	..	..	Trace	+	+	No great improvement, carbuncles not spread- ing.
19-5-1917	2.88	.117	.04	.263	.16	1 <sup>st</sup>	Trace	+	+	Still very toxicæmic and uræmic.
20-5-1917 to	..	..	..	..	..	..	N/d	+	N/d	Began to improve, car- buncles healing, less toxicæmic and general health better.
29-5-1917	..	..	..	..	..	..	N/d	+	N/d	Doing well in all respects.
30-5-1917	2.67	.039	.04	.128	.14	1 <sup>st</sup>	N/d	N/d	N/d	

The patient left hospital on the 12th June, 1917, fairly well. The carbuncles were practically healed and the urine was free from albumen and sugar. This patient was still alive and fairly well in November 1918.

Diabetic No. CLXII.—C. H., Hindu male, aged 50. There is a history of glycosuria, for 5 years. The patient was admitted in a very serious condition. There was slight glycosuria, marked albuminuria and a sloughing gangrene of the penis.

No attempt was made to treat the glycosuria by dieting as the patient was very ill and it was more a matter of combating the toxæmia and uræmia than curing his slight glycosuria.

His diet throughout was largely milk, sago, vegetables and stimulants.

The patient was admitted on the 26th May, 1917, and died on the 4th July, 1917. His death clinically was due to toxæmia and septic absorption from the gangrenous condition of the penis. He got restless, semi-conscious and gradually sank into coma. This is a typical example of the manner in which an end comes in these cases of old diabetics when gangrene and necrosis set in. The urine is examined and sugar and acetone found to be present; the diagnosis of diabetic coma is made.

Reference to our figures for the chemical analyses of the blood will show that death was really due to uræmia—the nitrogenous waste-products increased very greatly as the patient grew worse until eventually the ratio  $\frac{\text{N.P.N.}}{\text{T.N.}} \frac{1}{14.6}$  was present.

There were only traces of acetone in the urine for a few days before he died and the urine never showed any signs of di-acetic acid.

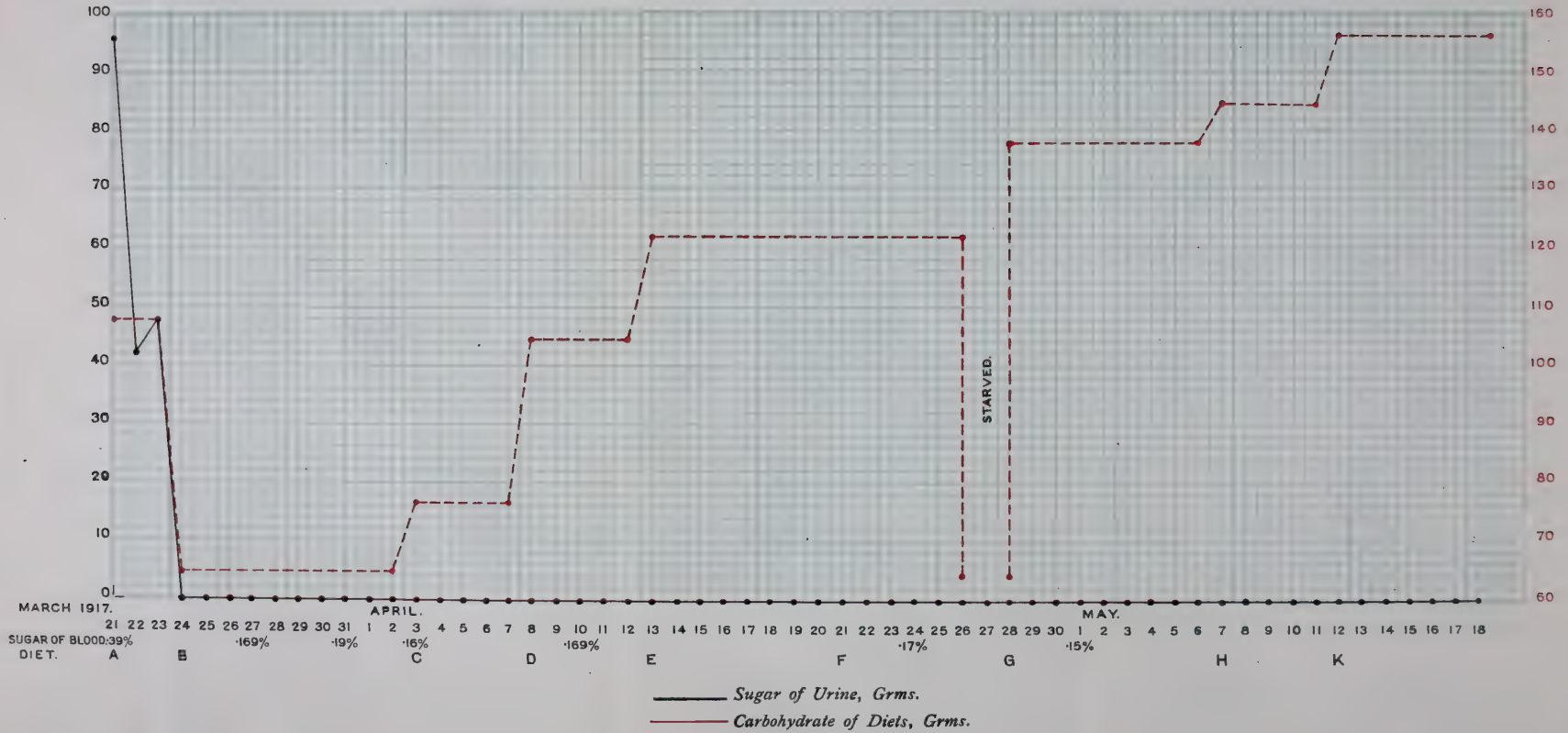
Date.	T. N. of blood. %	N. P. N. of blood. %	Urea of blood. %	Glucose of blood. %	Fat of blood. %	$\frac{\text{N. P. N.}}{\text{T. N.}}$	Sugar of urine.	Albumen of urine.	Acetone of urine.	REMARKS.
26-5-1917	3.06	0.47	0.42	.24	.16	$\frac{1}{65}$	Nil	+++	Nil	Condition bad.
27-5-1917 to 4-6-1917		..	..	..	..	..	Trace	+++	Nil	Began to improve at first but after a few days his condition changed for the worse.
5-6-1917	2.24	.061	.042	.32	.08	$\frac{1}{37}$	Trace	++	Nil	Very toxicemic.
6-6-1917 to 15-6-1917	..	..	..	..	..	..	Trace	+++	Nil	Getting worse. Urine scanty.
16-6-1917	2.46	1.68	.027	.22	..	$\frac{1}{14.6}$	Nil	+++	Nil	Sinking, very toxicemic and uræmic.





# CHART X.

## DIABETIC No 169



After this date the patient rallied for some days and he looked brighter and less toxæmic and uræmic, but the improvement was only temporary and he sank gradually into coma, dying on the 4th July, 1917.

We shall conclude the record of evolution of the treatment of the type of diabetes met with in India by giving the results of the investigations carried out on one of the most obedient and reliable patients we have had to deal with during this enquiry.

Diabetic No. 256.—Hindu male, aged 42 years. He gave a history typical of the condition. Some years ago he became very fat, later was found to be glycosuric and during the last six months he has been gradually losing weight. There is a family history of the disease. He was a clerk and led a sedentary life.

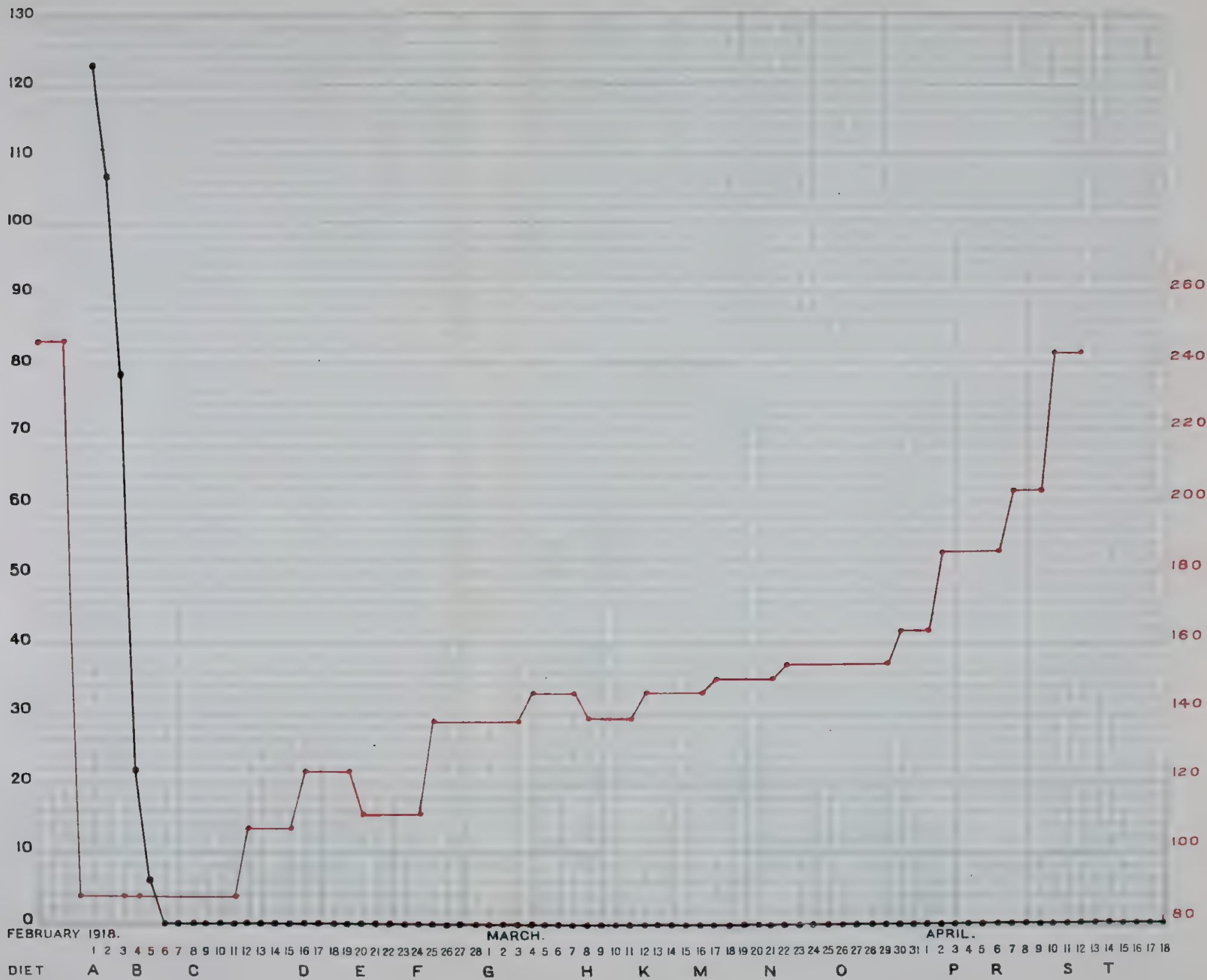
Admitted 1st February, 1918, and discharged on the 16th April, 1918.

It will be sufficient to give averages of the pooled figures for the different dietary periods.

Date.	Quantity of urine. c.c.	Sugar of urine. grms.	Diet.	Grms.
1-2-1918 to 3-2-1918	1700	103	A { Milk 1 sr. Bread 12 oz. Butter 2 oz.	P— 68.4 C— 247.0 F— 107.3
Trial diet.				
4-2-1918 5-2-1918 6-2-1918 7-2-1918	1700 1600 1700 1600	21.6 4.8 Nil Nil	B { Milk 1 sr. Vegts. 16 oz. Butter 2 oz.	P— 47.6 C— 87.8 F— 102.0
Protein and fat increased.				
8-2-1918 to 15-2-1918	2090	Nil	C { Do. plus Eggs 2	P— 51.6 C— 87.8 F— 107.0
Carbohydrate increase.				
16-2-1918 to 19-2-1918	1950	Nil	D { Do. plus Bread 1 oz.	P— 54.1 C— 104.8 F— 108.2
A further increase.				
20-2-1918 to 23-2-1918	1480	Nil	E { Do. plus Bread 1 oz.	P— 56.6 C— 121.8 F— 109.4

# CHART XI.

DIABETIC No. 256



— *Sugar of Urine, Grms.*  
— *Carbohydrate of Diets, Grms.*

Date.	Quantity of urine. c.c.	Sugar of urine. grms.	Diet.	Grms.
A decrease all round.				
24-2-1918 to 28-2-1918	1100	Nil	F { Milk 16 $\frac{3}{4}$ sr. Vegts. 16 oz. Butter 2 oz. Bread 2 oz. Eggs 2	P—46.9 C—111.0 F—99.7
Carbohydrate increased.				
29-2-1918 to 7-3-1918	1100	Nil	G { Do. plus Vegts. 4 oz. Bread 1 oz.	P—51.8 C—138.2 F—102.2
A general increase.				
8-3-1918 to 11-3-1918	1450	Nil	H { Milk 3 sr. Vegts. 16 oz. Butter 3 oz. Bread 4 oz. Eggs 3	P—53.9 C—145.0 F—117.3
A protein increase, carbohydrate decrease.				
12-3-1918 to 15-3-1918	1250	Nil	K { Milk 3 sr. Vegts. 8 oz. Butter 2 oz. Bread 3 oz. Eggs 2 Curds 4 oz.	P—69.6 C—139.6 F—114.0
A carbohydrate increase.				
16-3-1918 to 20-3-1918	1510	Nil	M { Do. plus Potatoes 1 oz.	P—70.2 C—144.7 F—114.4
Further increase in carbohydrates.				
21-3-1918 to 25-3-1918	1420	Nil	N { Do. plus Potatoes 1 oz.	P—70.8 C—149.8 F—114.8
Still further increase in carbohydrates.				
26-3-1918 to 2-4-1918	1620	Nil	O { Do. plus Potatoes 1 oz.	P—71.4 C—154.9 F—115.2
Protein decrease, carbohydrate increase.				
3-4-1918 to 5-4-1918	2225	Nil	P { Milk 3 sr. Vegts. 8 oz. Butter 2 oz. Bread 3 oz. Rice 2 oz. Eggs 4 Potatoes 2 oz. Fish 4 oz.	P—62.7 C—161.9 F—104.4
Carbohydrate increase.				
6-4-1918 to 10-4-1918	1750	Nil	R { Do. plus Rice 1 oz.	P—64.5 C—184.5 F—104.7

Date.	Quantity of urine. c.c.	Sugar of urine. grms.	Diet.	Grms.
Carbohydrate increase.				
11-4-1918 } to 13-4-1918 }	2180	Nil	S { Do. plus Bread 1 oz.	P- 67.0 C-201.5 F-105.8
General increase all round.				
14-4-1918 } to 16-4-1918 }	2300	Nil	T { Milk $\frac{3}{4}$ sr. Vegts. 8 oz. Butter 2 oz. Bread 6 oz. Rice 3 oz. Eggs 4 " Potatoes 3 oz. Fish 5 oz.	P- 74.8 C-241.1 F-107.9

The last diet was increased very much more quickly over the previous diet than we advocate, but the patient was anxious to go away and we wanted to estimate how much carbohydrate he could tolerate. He was advised to cut down the bread and rice and increase the vegetables as soon as he arrived home.

This was a very successful case and shows very clearly the method of re-educating the tissues to utilize carbohydrates.

#### THE METHOD OF TREATMENT OUTLINED.

The records, published above in some degree of detail, of fifteen cases that have been treated in the wards of the Medical College Hospital, Calcutta, give a very fair picture of the gradual evolution of the method of treatment which we have found to be most successful.

Large numbers of cases might be included in this paper but this is unnecessary as, except for slight individual idiosyncrasies, cases of the type of diabetes prevalent in India practically all react to proper dietetic treatment in the same manner. Many other cases were treated on different lines, and many different food-materials were experimented with, but it would cause this paper to reach an inordinate length to include the results obtained in this publication. Similarly, the effects of certain drugs and extracts were investigated but these results must also be excluded from the present paper. We may also state that outside the hospital large numbers of patients have been treated on the lines indicated above, from 1915 onwards to the present time, with very great success.



The method of treatment, which we recommend for the type of diabetes met with in India, is as follows :—

1. On first coming under observation, after attending to the bowels or anything likely to complicate the treatment, the patient is placed on a diet of milk, bread and butter, valued roughly to be worth 200–250 grammes of carbohydrate—the protein and fat being also fairly low.

The urine is collected and measured daily ; it is examined for albumen, sugar, acetone, ammonia and di-acetic acid. The average excretion of sugar for three or four days is thus determined and a very close guess can be made, from the amount of sugar excreted, as to the degree of the patient's tolerance to carbohydrates.

2. The diet is then changed and simply milk, vegetables and butter given for a few days. One to two pints of milk, 12–16 oz. of green vegetables and two ounces of butter for three days will clear the urine of sugar and the blood of hyperglycæmia in 99 per cent of all cases. If necessary, the milk and vegetables can be further reduced but this is rarely needed.

3. Depending on the physical condition of the patient this low type of diet may be continued for days, specially if the patient is very fat, or may be added to and the building up commenced. It may be necessary at this or other stages to give one to two or more ounces of alcohol, if the patient is feeble or the pulse weak.

4. If possible, the sugar of the blood should be estimated at least before the building-up process begins. If this is not feasible, then one day of carbohydrate starvation may be ordered at the end of the low dietary period.

The urine being free from albumen and there being no signs of nitrogenous retention of waste-products, we usually add to the low diet two eggs or four ounces of fish. This increases the protein and fat slightly without changing the amount of the carbohydrate. After a few days of a diet of this type—

Milk	..	1½ pints	} P = 50 grms. C = 87 .. (about). F = 100 ..
Green vegts.	..	16 oz.	
Butter	..	2 oz.	
Eggs	..	2	
or			
Fish	..	4 oz.	

we usually increase the green vegetables to 20 oz. This causes an increase in the carbohydrates by 11 grms. without changing the fat or protein to an appreciable extent.

5. The patient's diet is now worth just under 100 grammes of carbohydrate. Europeans will demand bread, Indians usually rice. Again depending on the severity of the case and the physical condition half an ounce of bread is given or more often we order four ounces of curd to be added to the diet. (The curd is sliced across in different directions and allowed to soak for two hours in water so that all the lactose dissolves out.)

This brings the diet up to, with 4 oz. curds,

Protein	..	70	grms.
Carbohydrate	..	98	"
Fat	..	115	"

6. After several days of this diet, half an ounce of bread is substituted for four ounces of vegetables. The diet is now worth—

Protein	..	69	grms.
Carbohydrate	..	96	"
Fat	..	115	"

In a few days another half ounce of bread may be added when the value becomes—

Protein	..	70.5	grms.
Carbohydrate	..	104.8	"
Fat	..	115.6	"

7. It is now best to reduce the protein and fat by decreasing the quantity of milk or curd—the carbohydrates may at the same time be increased by giving two ounces of potatoes. For instance, if the four ounces of curd are cut out and two ounces of potatoes added, the diet becomes—

Milk	..	1½	pints	} P- 58 grms., C- 115 " F- 108 "
Green vegts.	..	16	oz.	
Butter	..	2	oz.	
Bread	..	1	oz.	
Potatoes	..	2	oz.	
Eggs	..	2		

8. The patient still remaining free from sugar, after a few days of the above diet, the protein and fat may be increased by the addition of two eggs or four ounces of fish or chicken. Roughly the value will be—

Protein	..	62-65	grms.
Carbohydrate	..	115	"
Fat	..	112	"

9. This diet proving satisfactory a slight rise may be permitted in the carbohydrate element of the diet by the addition of one ounce of nuts or fruit. This will raise the carbohydrate element by about 6 grammes without changing the protein and fat to any appreciable extent, and it will help to vary the monotony.



10. As the patients usually become tired of large quantities of vegetables, it is well to cut down the amount at intervals, substituting less than the equivalent in carbohydrates of something more palatable. Thus, a complete change in the general character of the dietary often does good.

Milk	..	16	oz.	}	P- 59.5 grms. C- 88.4 .. F- 85.5 ..
Green vegts.	..	8	oz.		
Butter	..	1½	oz.		
Curds	..	2	oz.		
Eggs	..	4	oz.		
Fish or chicken	..	6	oz.		
Bread	..	2	oz.		
Potatoes	..	2	oz.		

That is, a decrease in all the constituents of the diet for a period during the building up process assists in enhancing the patient's tolerance.

This of course is absolutely essential if the analysis shows any tendency to an increase in the sugar content of the blood.

11. After four or five days on the new type of diet a fairly quick return may be made to the previous carbohydrate value.

Thus two ounces of Brazil nuts, or one ounce of pea nuts, and an ounce of bread may be added. The value thus becomes—

P—	63.5	grms.
C—	112	..
F—	88.5	..

12. The better class vegetables may now be used to some extent, which gives variety to the diet and at the same time raises the carbohydrate value. Thus a couple of slices of beetroot, a spoonful of cooked turnip or a carrot may be included amongst the vegetables.

No sudden increase in the value of the carbohydrates should be permitted; but, from day to day, a slight increase can be effected by varying the type of vegetable—the actual weight given remaining constant.

We give tables showing how easily this can be carried out in practice.

13. The building-up is continued by the addition of two ounces of potatoes, which will increase the carbohydrate value to about 125 grammes.

14. After a few days another half ounce of bread may be given, raising the diet all round to—

P—	69	grms.
C—	134	"
F—	90	"

15. With Indian patients the great craving is for rice; we usually, about this stage, cut down the carbohydrates by 20 grammes, in the

form of vegetables or bread, and substitute one-half ounce of rice, that is, decrease above diet by one ounce of bread and one ounce of potato and add half an ounce of rice :

Milk	.. 16 oz.	} P— 68 grms. C—123 " F— 88 "
Rice	.. $\frac{1}{2}$ oz.	
Green vegts.	.. 8 oz.	
Butter	.. $1\frac{1}{2}$ oz.	
Curds	.. 2 oz.	
Eggs	.. 4	
Fish or chicken	.. 6 oz.	
Bread	.. $2\frac{1}{2}$ oz.	
Potatoes	.. 3 oz.	

16. In a few days the whole ounce of rice may be given, raising the carbohydrates to 134 grammes.

17. The next step is to increase the carbohydrates in the form of vegetables—either by the addition of 4 ounces of green vegetables or a proportionally less quantity of other vegetables, such as for instance potatoes or partly potatoes and partly carrots, turnips, peas and beetroot.

The carbohydrate value of the diet will thus be increased to 145 grammes or so whilst the protein and fat remain almost the same.

18. Thus, by a slow gradual rise in protein and fat followed by a slight increase in carbohydrate ; this, in its turn, followed by a decrease in protein and fat and an increase in carbohydrates, the several constituents of the dietary are built up from the particular diet on which the urine of the patient was sugar-free.

The principle is a short step forward followed by a shorter step back, with, in severe cases, one day in the week a dietary carbohydrate-free. In this way the power of the diabetic to utilize carbohydrates is gradually regained and inside two months, in fairly average cases as seen in India, the dietary can be worked up to a level that will meet all the physiological needs of the body.

The above outline will serve to demonstrate the principle on which we have worked during the last four years. Each case has to be studied and treated on its merits. One patient may quickly be restored to a comparatively liberal carbohydrate diet ; another may react slowly and may require very careful dieting to prevent a return of the hyperglycæmia and glycosuria.

The fat flabby patients we reduce in weight by prolonging the periods of low feeding : the thin emaciated we try to build up as soon as possible and are quite pleased to observe a slight steady gain in weight.

The estimation of the sugar of the blood and daily examination of the urine are the two rocks on which the treatment is founded. Sugar should never be allowed to reappear. If it does, then we must return to a diet much inferior in carbohydrate value to that which the patient was consuming when the accident occurred.

In those cases where there is nephritis, retention of nitrogenous waste-products in the blood, threatening coma, carbuncles, gangrene, etc.,—several cases of this nature have been included in the fifteen described—the same general principles of treatment may be followed. If carefully carried out, success may be expected. In nephritis and threatening uræmia a low protein diet is also indicated until the flow of the urine is free and the ratio  $\frac{\text{N.P.N.}}{\text{T.N.}}$  of the blood is at least 1 : 80.

The blood should be taken several hours after a meal, so that the findings may not be complicated by the presence of amino-compounds absorbed from the alimentary canal.

We shall now give tables showing the values of certain suitable quantities of common food materials in proximate principles. By means of this table the value of a diet in grammes of proximate principles may be easily and readily calculated.

TABLE I.

*Approximate food values in grammes of certain specified quantities of the common food materials of Bengal.*

	Protein.	Carbohydrate.	Fat.	Calories.
1. Rice, 1 ch. or 2 oz. . . . .	4	47	5	208
2. Flour (maida), 1 ch. or 2 oz. . . . .	6	44	1	208
3. Flour (atta), 1 ch. or 2 oz. . . . .	7	40	1.5	204
4. Bread, 1 ch. or 2 oz. . . . .	6	36	..	180
5. Meat, 2 oz. . . . .	12	..	4	80
6. Bacon, 2 oz. . . . .	10	..	30	310
7. Fish, 4 oz. . . . .	22	..	4	120
8. Eggs, 2 .. . . .	4	..	5	61
9. Milk, $\frac{1}{2}$ sr. or 16 oz. . . . .	16	32	16	320
10. Butter and ghee, 1 ch. or 2 oz. . . . .	..	..	48	432
11. Mustard oil, 1 ch. or 2 oz. . . . .	..	..	58	522
12. Potato $\frac{1}{2}$ ch. or 1 oz. . . . .	1	6	..	30
13. Chana, $\frac{1}{2}$ pow or 4 oz. . . . .	29	5	3	146
14. Orange, 1 .. . . .	..	10	..	40
15. Plantain (Champa), 1 .. . . .	1	9	..	40
16. Broth, 4 oz. . . . .	3	..	..	12
17. Mung juice, 1 pow or 8 oz. . . . .	6	4	..	18
18. Massur juice, 1 pow or 8 oz. . . . .	6	4	..	40
19. Coconut water of 1, about 12 oz. . . . .	2	10	..	88
20. Green vegetables, 1 pow or 8 oz. . . . .	2	8	Trace	40

One chittack (ch.) equals 2 oz. (approximately).  
One pow equals 4 chs. or 8 oz.

In the treatment of diabetes in India the value of green vegetables cannot be too highly appraised. Bengal—the home of diabetes—is particularly fortunate in possessing an abundance of almost every variety of vegetables grown in the tropics or even in temperate climates. The Bengali also, and particularly the better-fed classes who are specially liable to fall victims to the disease, are able to prepare most palatable and appetising dishes from vegetables.

Large numbers of vegetables, the majority of which are quite unknown to the ordinary European, are made use of by the Bengali and many of these are exceedingly valuable in the treatment of diabetes.

In order to be in a position to open up this useful field and place the physician at his ease when asked by patients if they may eat this or that vegetable, we have taken the trouble to analyse practically every one of those that could be obtained. So far as we know, there is at present no information available on the subject.

In the following table we give the values of these vegetables in grammes of proximate principle per ounce.

We have placed them in their order of merit as regards the low carbohydrate value.

As will be seen, there are twenty-one different types of vegetables in common use whose carbohydrate value is less than one gramme per ounce.

TABLE II.

*Food values of the common green vegetables of Bengal, stated in grammes per ounce,*

	Protein.	Carbohydrate.	Fat.	Calories.
1. Puin Shak .. .. .	1.6	Trace	.08	1
2. Notay Shak .. .. .	.2	do.	Trace	..
3. Palang Shak .. .. .	.18	do.	do.	..
4. Uchehey .. .. .	.1	do.	do.]	..
5. Jhinga .. .. .	.1	do.	do.]	..
6. Papaya (green) .. .. .	.16	.1	.4	1
7. Lau .. .. .	.16	.27	.71	8
8. Chal-kumra .. .. .	.05	.36	Trace	2
9. Potal .. .. .	.21	.37	do.	3
10. Lettuce .. .. .	.39	.42	.39	5
11. Cauliflower .. .. .	.5	.45	.2	5
12. Barbati .. .. .	1.05	.51	.36	8
13. Moola .. .. .	.18	.54	Trace	3
14. Brinjal (Bagoon) .. .. .	.16	.57	.08	4
15. Asparagus .. .. .	.68	.66	1	4
16. Mocha .. .. .	.08	.71	Trace	3
17. Thor .. .. .	.01	.74	do.	2
18. Spinach .. .. .	.63	.78	1.2	5
19. Cabbage .. .. .	.28	.8	Trace	4
20. Cucumber .. .. .	.24	.93	.05	5
21. Celery .. .. .	.33	1	.03	5

The common better-class vegetables of Bengal, those with a carbohydrate value ranging from one to five grammes per ounce of vegetable, are given in the following table.

TABLE III.

*Food value of the common better-class vegetables of Bengal, stated in grammes per ounce.*

	Protein.	Carbohydrate.	Fat.	Calories.
1. Tomatoes.. ..	24	1.05	.14	5
2. French-bean .. ..	81	1.5	.35	12
3. Dhanrose (Ladies' finger) ..	57	1.7	.33	12
4. Leeks .. ..	36	1.7	.15	9
5. Mushroom .. ..	1.05	2.0	.12	13
6. Carrot .. ..	33	2.18	.12	11
7. Beet-root .. ..	69	2.2	.03	11
8. Turnip .. ..	39	2.4	.06	11
9. Beet .. ..	60	2.9	.03	14
10. Mankachu .. ..	07	3.3	Trace	13
11. Onion (green) .. ..	3	3.3	.03	13
12. Nol-kole .. ..	26	3.3	.16	15
13. Ole .. ..	68	3.8	.86	25
14. Parsnips .. ..	18	4.0	.15	19
15. Boiler green pea .. ..	2.0	4.3	.02	34
16. Artichoke .. ..	78	5.0	.06	23

Many of the varieties of fruit, indigenous to Bengal, are exceedingly useful in small quantities to add relish to an otherwise tasteless dish or to vary the monotony of an entirely vegetable diet. By means of the following table a small specified quantity of one of the fruits may be permitted, or may be substituted for a given quantity of some other food material, and the physician will know exactly what he is doing without having to rely on the usual empirical guess.

TABLE IV.

*Food values of some of the common fruits in Bengal, stated in grammes per ounce.*

	Protein.	Carbohydrate.	Fat.	Calories.
1. Blackberry .. .. .	41	1.02	.036	5
2. Footee .. .. .	39	1.1	Trace	5
3. Golap-jam .. .. .	37	1.4	.012	7
4. Lichee .. .. .	84	1.9	.07	11
5. Kharreja .. .. .	59	1.9	Trace	10
6. Dalim (Pomegranate) .. .. .	18	1.9	..	9
7. Pine-apple .. .. .	17	2.1	Trace	9
8. Bedana .. .. .	29	2.2	..	9
9. Peaches .. .. .	21	2.8	.03	12
10. Orange .. .. .	24	3.4	.06	15
11. Apricots .. .. .	3	4.0	..	17
12. Plum .. .. .	03	4.1	..	17
13. Champa-kala .. .. .	54	4.2	.039	19
14. Pears .. .. .	18	4.2	.15	18
15. Apples (ordinary) .. .. .	12	4.2	.15	18
16. Kantali-kala .. .. .	39	4.8	Trace	20
17. Apples (Kula) .. .. .	18	5.0	.14	21

Of course, when prescribing any of the above fruits, it is well to remember that the majority contain a small percentage of free sugar, so they should only be used with care and usually in great moderation.

We now give a general table of the results of the analyses of all the food materials of Bengal.

For the convenience of the prescribing physician these have been calculated on the basis of grammes per ounce and should prove useful to those dealing with diabetes or other diseases where a knowledge of the value of a diet in proximate principles is important.

TABLE V.

*Proximate principles in grammes per ounce of common Bengal food materials.*

	Protein.	Carbohydrate.	Fat.	Calories.
1. Rice—deshi .. .. .	1.9	25.6	.24	104
2. Banktulshi - atap .. .. .	2.1	24	.23	106
3. Banktulshi - sidha .. .. .	2	24.5	.28	108
4. Rice - Balam .. .. .	2	23	.12	101
5. Durbhanga Rice .. .. .	3	22.8	.3	106
6. Dadghani Rice .. .. .	1.6	24	.04	102
7. Wheat .. .. .	4	20	.7	103
8. Flour—maida .. .. .	3.3	21.3	.6	103
9. Flour—atta .. .. .	3.45	20.13	.87	102
10. Jaur - atta .. .. .	2.3	20.4	.6	96
11. Bajra - atta .. .. .	2.16	21.4	.5	98
12. Makai - atta .. .. .	2.9	19.8	.6	96
13. Suji .. .. .	4.2	14.2	.68	80

TABLE V.—(Contd.)

	Protein.	Carbohydrate.	Fat.	Calories.
14. Sattoo (gram)	7.6	17.1	55	103
15. Makhna ..	2.7	21.6	3	100
16. Barley ..	2.4	22.9	5	105
17. Buck-wheat ..	2.6	22.7	3	104
18. Pearl Barley ..	2.19	22.7	3	101
19. Oatmeal ..	3.8	19	1.7	106
20. Paniphal palo ..	2.5	22.5	Trace	100
21. Sati palo ..	1.68	21.6	do.	93
22. Arrowroot ..	2.4	25	do.	101
23. Muri ..	1.45	25	do.	105
24. Chalbhaja ..	1.3	23	do.	97
25. Khai ..	1.7	22.8	do.	98
26. Sona Mungdal ..	6.9	16.5	.78	100
27. Krishna Mungdal ..	6.3	16.2	.7	96
28. Mashkala dal ..	6.6	17.4	.33	99
29. Massur dal ..	7.4	16.5	.9	104
30. Motor dal ..	6.6	16.1	.57	96
31. Chola (gram) dal ..	5.7	15.3	1.3	95
32. Khensari dal ..	9.5	16.1	.27	104
33. Arhar dal ..	6.5	16.2	.99	100
34. Pun Shak ..	1.6	Trace	.08	1
35. Notay Shak ..	.2	do.	Trace	..
36. Palang Shak ..	.18	do.	do.	..
37. Uchehey ..	.1	do.	do.	..
38. Jhinga ..	.1	do.	do.	..
39. Papaya (green) ..	.16	.1	..	1
40. Lau ..	.16	.27	.7	8
41. Chal-kumra ..	.05	.36	Trace	2
42. Potal ..	.21	.37	do.	3
43. Lettuce ..	.39	.42	.39	5
44. Cauliflower ..	.5	.45	.2	5
45. Barbatl ..	1.05	.51	.36	8
46. Moola ..	.18	.54	Trace	3
47. Brinjal (Bagoon) ..	.16	.57	.08	4
48. Asparagus ..	.68	.66	1	4
49. Mocha ..	.08	.7	Trace	3
50. Thor ..	.01	.7	do.	2
51. Spinach ..	.63	.78	1.2	5
52. Cabbage ..	.28	.8	Trace	4
53. Cucumber ..	.24	.93	.06	5
54. Celery ..	.33	1	.03	5
55. Tomatoes ..	.24	1.05	.14	6
56. French-bean ..	.81	1.5	.35	12
57. Dhanrose ..	.57	1.7	.33	12
58. Leek ..	.36	1.7	.15	9
59. Mushroom ..	1.05	2.0	.12	13
60. Carrot ..	.33	2.18	.12	11
61. Beet-root ..	.69	2.2	.03	11
62. Turnip ..	.39	2.4	.06	11
63. Beet ..	.69	2.4	.03	14
64. Mankachu ..	.07	3.3	Trace	..
65. Onion (green) ..	.31	3.3	.03	14
66. Nol-kole ..	.25	3.3	.16	15
67. Ole ..	.68	3.8	.86	25
68. Parsnips ..	.48	4.0	.15	19
69. Boiler green pea ..	2.0	4.3	1.02	34
70. Artichoke ..	.78	5.0	.06	23
71. Potato, Bombay ..	.49	4.3	.15	20
72. Potato, deshi ..	.56	5.9	.18	27
73. Banga-alu ..	.47	6.7	.09	28
74. Saakalu ..	.46	6.3	.06	26



TABLE V.—(Concd.)

	Protein.	Carbohydrate.	Fat.	Calories.
75. Chubri-alu { .. .. .	27	4.8	Trace	20
76. Goori-kachu .. .. .	3	5.7	Trace	24
77. Kantal-bichi .. .. .	3.9	9.4	.59	57
78. Sheem .. .. .	37	2.1	.17	11
79. Motor shuti .. .. .	2.5	6.6	.3	39
80. Black berry .. .. .	41	1.02	.036	6
81. Footee .. .. .	39	1.1	Trace	7
82. Golap-jam .. .. .	37	1.4	.012	7
83. Lichee .. .. .	84	1.9	.07	11
84. Kharmuja .. .. .	59	1.9	Trace	9
85. Dalim .. .. .	18	.18	..	9
86. Pineapple .. .. .	17	2.1	Trace	9
87. Bedana .. .. .	29	2.2	..	9
88. Peaches .. .. .	21	2.8	.03	12
89. Orange .. .. .	24	3.4	.06	15
90. Apricots .. .. .	3	4.0	..	17
91. Plum .. .. .	03	4.1	..	17
92. Champa-kata .. .. .	54	4.2	.039	19
93. Pears .. .. .	18	4.2	.15	18
94. Apples (ordinary) .. .. .	12	4.2	.15	18
95. Kantali-kala .. .. .	39	4.8	Trace	20
96. Apples (Kulu) .. .. .	18	5.0	.14	21
97. Coconut .. .. .	1.1	6	16.8	190
98. Lemons .. .. .	12	2.9	.09	13
99. Figs .. .. .	45	5.6	..	24
100. Grapes .. .. .	39	5.7	.48	29
101. Banana .. .. .	39	6.6	.18	28
102. Mangoe, Bombay .. .. .	.036	5.2	.22	22
103. Kismis .. .. .	78	3.4	2.4	38
104. Dates (best) .. .. .	72	20.6	.57	90
105. Dates (country) .. .. .	3	16.2	.25	68
106. Rui fish .. .. .	5.1	..	2.2	40
107. Mirgel fish .. .. .	6	..	.1	24
108. Magur .. .. .	6.3	..	.6	30
109. Kai .. .. .	7	..	.8	35
110. Singi .. .. .	7.3	..	1.2	40
111. Chingri .. .. .	5	..	.14	21
112. Milk .. .. .	1.28	1.17	1	18
113. Chana .. .. .	6.6	.11	5.5	76
114. Dadhi .. .. .	1.4	.8	1	17
115. Goat's milk .. .. .	.7	1	.96	15
116. Mother's milk .. .. .	.42	.75	1.5	18
117. Cream (shar) .. .. .	.8	.8	8.1	79
118. Sandesh] .. .. .	5.4	12	6	75
119. Beef .. .. .	6	..	1	33
120. Bacon .. .. .	2.7	..	14.5	141
121. Goat's meat .. .. .	7.2	..	.73	45
122. Mutton .. .. .	4	..	10	106
123. Fowl .. .. .	7	..	.9	36
124. Poultry .. .. .	6	..	1.1	33
125. Raw meat juice .. .. .	6	..	..	2
126. Eggs (hen's) .. .. .	3.9]	..	3.3	45
127. Eggs (duck's) .. .. .	3.9	..	4.2	53
128. Brazil-nuts .. .. .	5.1	2.1	20	208
129. Beech-nuts .. .. .	3.9	2.3	10.2	116
130. Filbert-nuts .. .. .	4.5	3.9	19.5	209
131. Pistachios .. .. .	6.6	4.8	16.2	191
132. Walnuts .. .. .	4.8	4.8	19	209
133. Almonds .. .. .	6.3	5.1	16.5	194
134. Pea-nuts .. .. .	7.5	7.2	11.4	161
135. Chestnuts .. .. .	1.86	12.6	16.2	203

## CONCLUSIONS.

1. The onward march of glycosuria—from the pre-glycosuric stage, transient and intermittent stages to a permanent condition—can be reversed by prolonging the intervals between meals. Even severe cases of the Indian type of diabetes can be made sugar-free and the hyperglycæmia reduced to a normal glycæmia by a restricted dietary for a few days.

2. The glycosuria of India is a true diabetes from the beginning, *i.e.*, “it is a specific deficiency of the power of assimilating food” (Allen). The specific function of the internal secretion of the pancreas is below normal in those individuals who develop glycosuria.

3. We have brought forward conclusive evidence to show that the prevailing form of diabetes met with in India is of a very mild type—milder probably than the *diabetes levis* of European countries.

Patients rarely die from diabetic coma and the type shows little tendency to pass into the grave forms of the disease.

4. Reduction of the carbohydrate element below the level of the patient's tolerance is practically never followed by any signs of severe acidosis. A trace of acetone may occur in the urine, di-acetic acid has never once been found amongst thousands of urinary analyses, the ammonia content of the urine remains stationary; so that the fear of acidosis and coma was proved to be imaginary.

5. Having rid our minds of the fear of coma, there was nothing to force us to increase the carbohydrate element of the food against our wishes.

Patients were, therefore, kept on a low carbohydrate diet for several days or even weeks. The hyperglycæmia and the glycosuria disappeared. It was then found that slight increases in the value of the different proximate principles could be effected, without a return of the hyperglycæmia and glycosuria.

6. In this way during 1915—continuing the work begun on the same lines in 1913—the principle of the re-education of the tissues to utilize carbohydrates was evolved. This work was done quite independently of other workers on the same subject.

A series of fifteen cases are given in considerable detail to show the methods of treatment that were adopted. Charts of the results of the daily analyses and of the carbohydrate value of the diets are also given to illustrate the elimination of sugar from the urine and the gradual

raising of the patient's carbohydrate tolerance. As will be evident, no great difficulty was experienced in re-building up the carbohydrate tolerance to a level sufficient for the physiological requirements.

7. The daily analyses show the practical absence of all signs of acidosis. The amount of acetone excreted daily rarely exceeds the tenth of one gramme: the urinary ammonia seldom reaches one gramme: and di-acetic acid is never present. (In one or two severe types of diabetes in Europeans that we have had under observation, acetone and di-acetic acid were being freely excreted.) We have never been forced to give alkalis through fear of an increasing acidosis.

8. In none of the cases published in this paper was Allen's starvation method tried. After the receipt of his book we did experiment with his method; as will be seen from the analyses of the urine for acetone, di-acetic acid, etc., our own method was giving very satisfactory results, and we did not therefore feel justified in starving our cases for the sake of the slight gain in time of perhaps one day. Allen lays stress on the virtue of complete starvation as a means of preventing acidosis. As we never had any signs of acidosis or coma, starvation was unnecessary, and, as a practical asset in treatment, the Indian patient and others much prefer a diet of milk, green vegetables and a little butter to nothing at all.

The hyperglycæmia and glycosuria disappear in a few days, very nearly as quickly as when complete starvation is insisted on.

9. Some, amongst the fifteen cases published, are of great interest from other points of view besides the methods adopted in treating the diabetes.

Thus, Diabetic No. IX, who died in coma, shows very clearly the gradual failure in the elimination of nitrogen and the diminution in the quantity of urine excreted until practically complete suppression occurred. Death was due to uræmia.

Diabetic No. X, who also eventually died from septic absorption and uræmia, is of interest as one of the first cases in which we examined the blood for evidence of uræmia. The urea of the blood twelve days before death was 0·08 per cent (urease method). He also developed almost complete suppression as coma set in.

Diabetic No. CLXIX is of very great interest as illustrating the effect of dietetic treatment not only with regard to the management of a

case of diabetes, but also the very favourable influence that may be exerted on a patient in the early stages of uræmia by careful dieting.

The blood examination in this case (also cases Nos. CLXII and CCLXX) showed a very serious state of things. Besides the hyperglycæmia there were abundant signs of an accumulation of nitrogenous waste-products in the blood. The percentage of urea had increased to over double the normal and the relationship  $\frac{\text{Non-protein nitrogen}}{\text{Total nitrogen}}$  had very seriously departed from the normal.

In Nos. CLXIX and CCLXX the treatment adopted seemingly had the desired effect and the marked signs of uræmia gradually disappeared. No. CLXII, on the other hand, grew worse—nitrogenous retention of waste-products increased until, three weeks before death, the ratio of non-protein nitrogen to total nitrogen was 1 : 14·6, the normal being 1 : 120 or more—the patient developed uræmic coma and died.

Several of the cases illustrate the beneficial effects of dietetic treatment on the carbuncles, septic infections, gangrene, or other complications of diabetes.

10. We give the analysis of over one hundred food materials in common use in India and have arranged them in tables showing the values of their proximate principles in grammes per ounce of food-stuff.

By means of these tables the physician is able to calculate the value of any diet he wishes to prescribe in a few minutes. He is also able to substitute one food material for another and know exactly what the change means.

Bengal is exceedingly fortunate in possessing a large number of very palatable vegetables which possess a very low carbohydrate value. We have given a list of twenty-one which contain less than one gramme of carbohydrate per ounce.

There are probably others as we make no claim that the list is exhaustive.

The family cook is very expert in providing tasty curries of all sorts from these vegetables and different spices. With the addition of eggs or fish and butter or ghee a great variety of palatable dishes can be produced.

11. We give a form here which has been found very useful in the dieting of diabetics. These forms can be printed and copies given to

each patient. The physician can work out the carbohydrate value of the diet with the assistance of this form in a few minutes.

## TREATMENT OF DIABETES.

TABLE VI.

*Dietary Form.*

Green vegetables (low carbohydrate value) Table I			.. oz. × 1 =	Grms. of carbohydrate.
Vegetables (better class) Table II	..	oz. × 2½ =	..	..
Mixture of all types of vegetables	..	oz. × 2 =	..	..
Oatmeal .. ..	..	oz. × 19 =	..	..
Bread toast .. ..	..	oz. × 18 =	..	..
Potatoes .. ..	..	oz. × 6 =	..	..
Brazil-nuts .. ..	..	oz. × 2.1 =	..	..
Walnuts .. ..	..	oz. × 4.8 =	..	..
Pea-nuts .. ..	..	oz. × 7.2 =	..	..
Almonds .. ..	..	oz. × 5.1 =	..	..
Oranges .. ..	..	oz. × 10 =	..	..
Papaya .. ..	..	oz. × 5 =	..	..
Pears .. ..	..	oz. × 4.2 =	..	..
Pine-apple .. ..	..	oz. × 2.1 =	..	..
Apples .. ..	..	oz. × 5 =	..	..
Lichees .. ..	..	oz. × 1.9 =	..	..
Figs .. ..	..	oz. × 5.6 =	..	..
Peaches .. ..	..	oz. × 2.8 =	..	..
Lemons .. ..	..	oz. × 2.9 =	..	..
Bananas .. ..	..	oz. × 9.0 =	..	..
Milk .. ..	..	oz. × 2.0 =	..	..
Rice .. ..	..	oz. × 23.5 =	..	..
Wheat and all flours ..	..	oz. × 20 =	..	..
Dals, legumes .. ..	..	oz. × 16.5 =	..	..

# THE PATHOLOGY OF EXPERIMENTAL RABIES.

## I. KIDNEYS, ADRENALS, LIVER, PANCREAS, SPLEEN.

BY

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MOST of the work hitherto published on the pathology of rabies has dealt with changes in the central nervous system, and comparatively little attention seems to have been paid to other organs and to the general pathology of the disease.

It is not pretended that the present paper gives anything approaching a complete account of the pathological processes due to rabies in the organs it deals with: it merely speculates on the connection between some accumulated facts.

### GLYCOSURIA.

One of the outstanding features of laboratory rabies is the appearance of sugar in the urine. The fact has been known for decades but no satisfying explanation has been offered.

If sugar appears in the urine in any large quantity, there must have been previously an excess of sugar in the blood. Sugar in the blood is usually derived from the store of glycogen in the liver.

Glycogenolysis is accelerated by stimulation of the piqure centre when the adrenals are in a condition to perform their function. Excess of adrenalin in the blood excites the terminal apparatus of the

sympathetic nerves in the hepatic cells to produce glycogenolysis even when the nerve connections of the liver have been severed. If the adrenal nerves are severed, toxic and mechanical stimuli of the centre in the 4th ventricle are said not to produce glycogenolysis, for the reason that the glands are not excited to hypersecretion.

Products of the adrenal glands seem therefore to be intimately concerned in, if not actually essential to, the hydrolysis of the glycogen of the liver. The 'internal secretion' of the pancreas appears to exercise some control over glycogenolysis, but whether this control is direct or acts by way of the adrenal secretion is not known. Stimulation of the pituitary body, whether mechanical or toxic, leads to glycogenolysis just as does stimulation of the piqure centre. The secretion of the thyroid is also concerned in the production of glycosuria.

Glycosuria in rabies may therefore be due to—

(1) Stimulation of the diabetic centre in the 4th ventricle either by the growth of the rabies organism or by the toxic products of its metabolism. The irritated centre reacts on the adrenals and thereby on glycogen store of the liver. Deprivation of oxygen through defective respiratory movements may also stimulate the diabetic centre.

(2) Stimulation of the pituitary body in a similar way.

(3) Interference with the hypothetical control of the pancreas over glycogenolysis.

(4) Disturbance of some function of the thyroid.

With the fixed-virus now in use in this Institute rabbits generally present well-marked symptoms of rabies by the 5th day, they are generally lying paralysed on the 6th day and sometimes succumb that night. The majority are killed moribund on the 7th day. They will rarely last over till the 8th day.

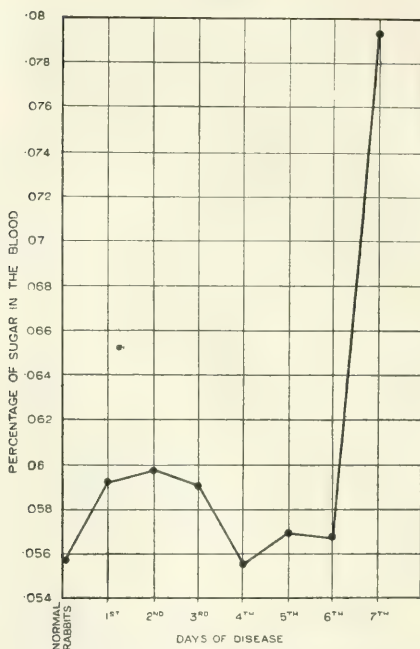
The following table shows the figures obtained in estimating the percentage of sugar in the blood of rabbits by the Benedict-Cambridge method. See also Chart I.

TABLE I.

				Mean values.	
				sugar in blood.	
29	Normal rabbits		..	·0558%	
23	1st day fixed-virus rabbits		..	·0592	" "
23	2nd " " "	..	..	·0598	" "
23	3rd " " "	..	..	·0590	" "
23	4th " " "	..	..	·0556	" "
30	5th " " "	..	..	·0569	" "
32	6th " " "	..	..	·0568	" "
31	7th " " "	..	..	·0794	" "



CHART 1.



It is clear that an increase of the sugar in the blood is a late phenomenon in laboratory fixed-virus rabies and that it does not occur until shortly before death.

Similarly, sugar does not appear in the urine until the 7th day and not always then. Its appearance depends somewhat on the vitality of the animal; the longer the rabbit survives after the onset of symptoms, the more likely is a considerable quantity of sugar to be found in the urine. It must also be remembered that cachexia in diabetics and the approach of death will often cause a diminution in the amount of sugar eliminated. The position in rabies appears to be that conditions become favourable for glycosuria but that the total quantity of glycogen hydrolysed is small owing to the rapid termination of life. Some estimations of the glycogen present in the liver of rabbits dying from rabies were made, but the departures from the normal average were too small to be of

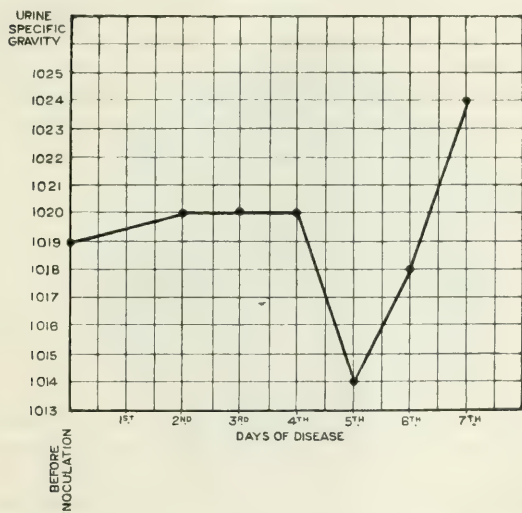
any account. As a rule not more than 0.25 gram of sugar finds its way into the urine during the last 24 hours of a fixed-virus rabbit's life, and often much less, so that one could scarcely expect to be able to demonstrate a diminution in the glycogen store of the liver, which is variable in the healthy animal.

*Other findings in the urine.*

The urine of herbivora in health is alkaline. The urine of fixed-virus rabbits becomes acid. No di-acetic acid has been found to accompany the sugar. The acidity is probably due to the fact that the animal has ceased to eat or assimilate vegetable food and is living on its tissues, since acids are produced in the physiological combustion of proteins within the organism.

Coagulable protein is frequently present in the urine towards the end. It consists chiefly of globulins, with little albumin.

CHART 2.



The specific gravity of the urine shows a marked drop on the 5th day of the disease, but rises considerably above the normal as sugar begins to be excreted. The figures from which Chart No. 2 was constructed are the averages of 20 observations on each day of the disease,

and were obtained by weighing the urine in a specific gravity bottle after clearing it by centrifugation.

The percentage of chlorides in the urine was also estimated daily by Volhard's method with the result shown in the following table. Twenty estimations were made for each average.

TABLE II.

Description of animals.	Average percentage of chlorides in the urine.
Normal rabbits	0.186
1st day fixed-virus rabbits	0.404
2nd " " " "	0.249
3rd " " " "	0.308
4th " " " "	0.327
5th " " " "	0.211
6th " " " "	0.224
7th " " " "	0.164

Not much information can be derived from these figures in the absence of exact data correlating the total quantity of urine passed and the respective weights of the animals. Still, on the 5th day there is a lesser percentage which coincides with the fall in the specific gravity on that day, and there is an indication that towards the end of the disease the output of chlorides is diminished which suggests renal insufficiency.

Excess of adrenalin in the blood stream is said to lead to a retention of chlorides.(1)

#### THE ADRENALS.

The *adrenal glands* of rabbits dying from fixed-virus rabies, or killed moribund on the 7th day, are usually enlarged, and their mean weight per kilo is 0.307 gram, whereas in normal rabbits their mean weight per kilo is 0.21 gram. The increased weight is probably chiefly due to engorgement of the vascular spaces in the glands.

Sections of the organs show that profound changes have taken place both in the cortex and in the medulla, but chiefly in the latter. A minute description of the histological appearances will serve no useful purpose; it will suffice to say that the cellular elements are not completely destroyed as may happen in severe toxic affections such as diphtheria, but appear normal in some parts and diseased in others. In the medullary portion the cytoplasm of the cells is affected more than the nuclei. Later, the nuclei become shapeless, and finally may

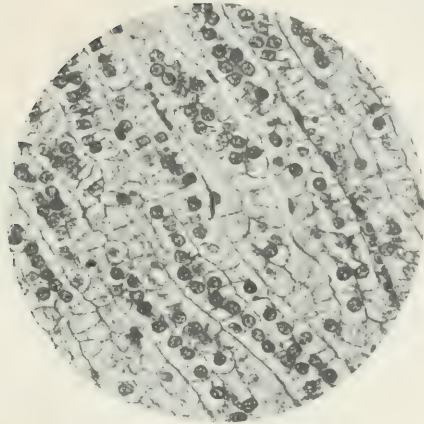


Fig. 1.—Adrenal cortex from a normal rabbit.

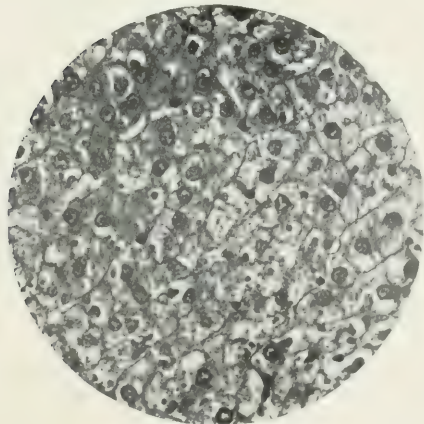


Fig. 2.—Adrenal cortex from a fixed-virus rabbit.

Appearance of the cortex of the adrenal of a rabbit, killed moribund with fixed-virus rabies.

Note the disappearance of all regularity in the columns of cells and the disintegration of both the cytoplasm and the nuclei of many cells.

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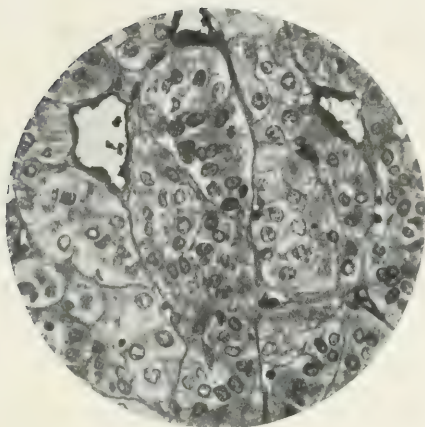


Fig. 3.—Adrenal medulla from a normal rabbit.

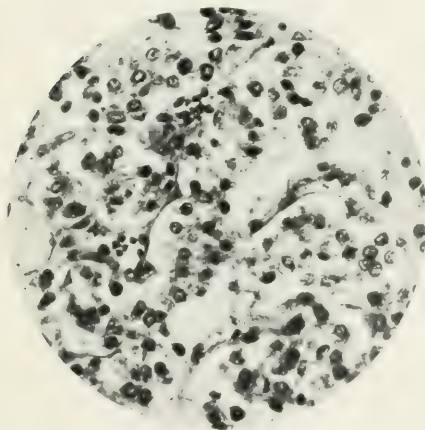


Fig. 4.—Adrenal medulla from a fixed-virus rabbit.

The medulla of the adrenal of a rabbit, killed moribund with fixed-virus rabies. Note the disintegration of the cytoplasm of the majority of the cells. The nuclei break up later.

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disappear leaving nothing but amorphous lumps and strands in the reticulum once occupied by healthy cells. The process is perhaps an autolytic one, the cells being disintegrated by their own ferments.

In the cortical area dissolution of cell cytoplasm is not so marked; the primary effect seems to be on the nuclei.

Photomicrographs 1, 2, 3, and 4, give an idea of the appearances of the adrenal glands of the rabbit in health and when dying from rabies.

Whatever the exact nature of the morbid changes may be, the result is that at least a portion of the gland ceases to perform its normal functions. We know that these functions are important and essential to life, and any morbid process which checks, even temporarily, the proper functions of the adrenal glands must therefore endanger life.

We do not know what the functions of the adrenals are, and we can only guess at the interrelation of the adrenals and other ductless glands, hence any observations on the condition of the adrenals and of their extracts, when a departure from normal is caused by disease, may be of value.

Having this in view, a series of estimations of the pressor effect of watery extracts of adrenal glands in fixed-virus rabies were made on sheep and kymographic tracings obtained.

CHART 3.

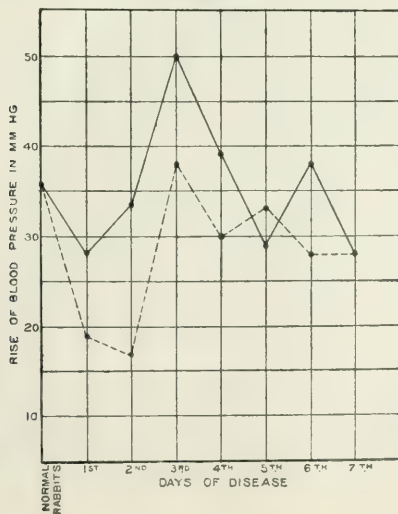


Chart No. 3 (continuous line) shows the mean rise of pressure in mm. Hg. resulting from the intravenous injection of the extracts of the adrenal glands from rabbits sacrificed on the different days of the disease. The curve starts from the mean pressor effect of extract of adrenals from normal rabbits. Sheep of approximately the same weight were used in each experiment.

The adrenal glands vary in size even in health and the adrenalin content is not directly proportional to the variation in weight of the gland, so the crude figures from which the curve alluded to above was plotted need correction for the weight of glandular substance employed in preparing the extracts for injection. When this correction is applied a second curve is obtained (Chart 3 broken line) which repeats the form of the first with slight modifications. From these two curves it may be deduced that the adrenalin content of the glands, as measured by its pressor influence, is lessened during the 48 hours succeeding the subdural inoculation with fixed-virus, that a recovery takes place and that towards the end of the disease there is a diminution in the pressor influence of the adrenal extracts.

It has been noted by T. R. Elliott that injury to the brain causes a definite fall in the adrenalin content of the glands, so it is probable that the initial fall mentioned above is directly dependent on the cranial operation. In spite of the somewhat gross lesions of the adrenal gland seen in microscopical sections, it is clear that the quantity of pressor substances obtained by extraction with physiological salt solution is not in great defect. Nevertheless, it does not follow that the affected glands were adding their secretion to the blood stream normally either in quantity or quality. As a check on the physiological method a new series of estimations was made by the chemical method described by Folin, Cannon and Denis.(2) Whether the method indicates the actual quantity of adrenalin, as its originators claim, is not of much importance in the present enquiry. A sufficiently large number of observations has been made to eliminate accidental errors, the results are comparable and conclusions can be drawn.

To have followed up the variations in the adrenalin content of the glands on the different days of the disease would have required a larger number of animals than could be spared, and so comparisons between normal rabbits and rabbits killed or dying on the 7th day only were made.

Table III shows the differences in the weights of the adrenals in healthy and rabid guinea-pigs and rabbits.

TABLE III.

Description of animals.	Mean weight of adrenals per kilo of body-weight.
1. Normal rabbits .. ..	0.21 gram.
2. Fixed-virus rabbits (subdural inoculation) ..	0.307 ..
3. Normal guinea-pigs .. ..	1.05 ..
4. Street-virus guinea-pigs (intramuscular injection)	1.50 ..

It will be noticed (1) that the adrenals of the guinea-pig are about 5 times heavier than the adrenals of the rabbit, though the former animal is much smaller; (2) that the mean weight of the adrenals per kilo of body-weight is increased by about one-third in both species of animal in consequence of infection with rabies.

Table IV gives the mean weight of adrenalin per gram of gland substance and per kilo of body-weight in the different classes of animals tested.

TABLE IV.

Description of animals	Grams adrenalin per gram of fresh gland substance.	Grams adrenalin per kilo of body-weight.
1. Normal rabbits .. ..	.001595	.000301
2. Fixed-virus rabbits, killed moribund 7th day	.000972	.000262
3. Fixed-virus rabbits, died 6th night or 7th morning .. ..	.000758	.000235
4. Normal guinea-pigs .. ..	.000478	.000371
5. Street-virus guinea-pigs, dissected soon after death .. ..	.000295	.000450
6. One guinea-pig. Died from acute septic infection .. ..	.000061	.000155
7. One fixed-virus rabbit. Smelling when dissected .. ..	Nil	Nil

The following points are brought out by Tables III and IV :—

(1) The mean weight of the adrenals in fixed-virus rabbits is 1.46 times the mean weight of the adrenals of normal rabbits. The adrenals of fixed-virus rabbits contain 0.6 of the quantity of adrenalin per gram of gland found in normal rabbits.

The total quantity of adrenalin present in the fixed-virus rabbits was therefore about 0.87 of the quantity found in normal rabbits. The physiological method gave about 0.92, so there is a fair agreement between the two.

(2) The adrenalin present in the glands begins to diminish soon after death.

(3) The mean weight of the adrenals of street-virus guinea-pigs is 1.42 times the mean weight of the adrenals of normal guinea-pigs, and the street-virus glands contain 0.61 of the quantity of adrenalin per gram of gland found in normal glands.

The total quantity of adrenalin present in street-virus guinea-pigs is therefore about 0.87 of the quantity found in normal guinea-pigs.

(4) The difference in the ratio of adrenalin per kilo of body-weight in rabbits and guinea-pigs

Normal rabbits	..	..	0.003301	= 1.15
Fixed-virus rabbits	..	..	0.002262	
Normal guinea-pigs		..	0.003371	= 0.82
Street-virus guinea-pigs		..	0.004450	

is due to the greater proportionate loss in weight in the guinea-pigs during the course of the disease.

(5) As is well known, acute septic infections cause a great fall in the adrenalin content of the glands. The onset of putrefaction has a similar effect. Nos. 6 and 7 in Table IV are merely inserted as illustrations of these facts.

The physiological experiments, already detailed, show that during the course of fixed-virus rabies in rabbits there are variations in the pressor effect of extracts of the adrenal glands and that towards the termination of the disease the pressor effect is definitely though slightly diminished. A similar result was reached by employing a chemical method. Porak(3) arrives at the conclusion 'that, under the influence of the rabic virus,—at the terminal period of the infection,—the functional activity of the adrenals, manifested by the action of adrenalin, is much diminished.' Earlier in his paper he states that during the terminal period the pressor action is often *nil*. There are certain



PLATE III.

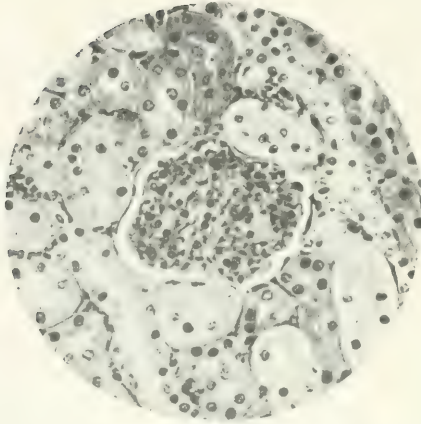


Fig. 5.—Kidney from a normal rabbit.

A glomerulus and secreting tubules from the kidney of a normal rabbit.

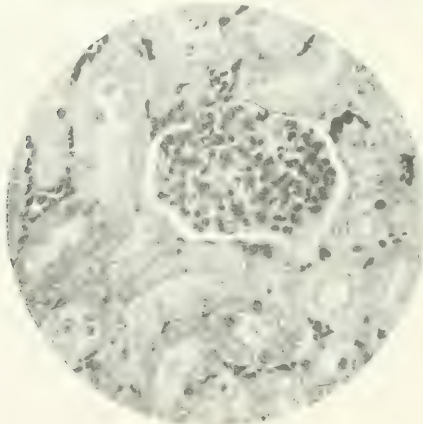


Fig. 6.—Kidney from a fixed-virus rabbit.

A glomerulus and secreting tubules from the kidney of a rabbit, killed moribund with fixed-virus rabies.

Note some cloudiness of the glomerulus. The principal change is in the tubules. The nuclei fail to stain properly and the cells are swollen, granular and exfoliating.

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differences in procedure which may account for his arriving at a conclusion somewhat at variance with mine. Porak used a fixed virus which allowed his rabbits to survive till the 11th or 12th day. My fixed virus does not generally permit survival beyond the end of the 7th day. We both sacrificed the experimental animals when moribund. The longer survival of Porak's animals may have been accompanied by greater destruction of the adrenal tissue.

Porak dried his glands in vacuo over sulphuric acid, and pounded them and made his extracts at some later period. My extracts were all quite freshly prepared and used without delay, so that there could have been no appreciable loss in adrenalin.

The fact remains that both in fixed-virus and in street-virus rabies there is extensive damage to the adrenal glands, and their content of adrenalin is diminished in a greater or less degree. How this damage is caused is another matter, but the presence of some toxic agent in the blood stream is certainly indicated. It is not shown, however, that in spite of this diminution and cellular damage an excess of adrenalin had not entered the blood stream.

#### THE KIDNEYS.

The *kidneys*, as might be suspected from the presence of globulins in the urine, are not unaffected in fixed-virus rabies. The principal damage occurs to the cells of the secreting tubules, which become granular and lose their outline. The nuclei of the cells fade away, some disappearing altogether, and many of the remainder are only faintly coloured by stains.

Evidently part of the secreting tissue of the kidneys is disintegrating under the influence of some toxic agents in the blood stream.

The kidneys in normal rabbits weigh 7·7 grams per kilo and in fixed-virus rabbits 8·5 grams per kilo, so in rabies there is evidence of congestion of these organs—*vide* photomicrographs 5 and 6.

#### THE PANCREAS.

The *pancreas* does not appear to be grossly affected when examined by ordinary histological methods, but it is improbable that it resists altogether the influences which cause such marked lesions in the adrenals and kidneys.



## THE LIVER.

The *liver* parenchyma does not appear to be appreciably altered. In normal rabbits the livers weighed 36·14 grams per kilo, and in a long series of fixed-virus rabbits 37·14 grams per kilo, so there is possibly a slight engorgement of the organ.

## THE SPLEEN.

In normal rabbits the *spleens* weighed 0·35 gram per kilo and in fixed-virus rabbits 0·30 gram per kilo. The difference is not material. If the spleen is affected at all, it is on the side of atrophy.

\*       \*       \*       \*       \*       \*       \*

Incidentally, it may be remarked that the colorimetric method of estimating adrenalin described by Folin, Cannon and Denis should be employed with due caution, bearing in mind that the coloration observed may not be entirely due to adrenalin. The authors of the method call attention to the fact that the uric acid contained in the blood and other organs can produce a colour similar to that given by adrenalin, but they state that the colour is so faint that it can be disregarded.

I do not agree that it can be entirely disregarded, for the figures in the subjoined table show that even in a normal animal one-fourth of the coloration given by the adrenals may be obtained from an equal weight of spleen substance.

TABLE V.

*Showing the actual relations of the different depths of colour given by equal weights of organs from a normal rabbit.*

Adrenals	..	..	116
Spleen	..	..	29
Liver	..	..	20
Lung	..	..	8
Kidney	..	..	4
Brain	..	..	3
Muscle	..	..	2
Blood	..	..	2

As the organs vary, even when obtained from normal animals, in the amount of colour-producing substances such as uric acid, phenol derivatives and perhaps others yet unknown, which their extracts contain, it is legitimate to suppose that greater variations may occur in disease.

## SUMMARY.

1. Hyperglycæmia is a late phenomenon in fixed-virus rabies. It may or may not be followed by glycosuria.

2. If a reduction in the glycogen contained in the liver occurs, it is too small to be measured with accuracy.

3. The urine becomes acid and globulins are excreted with but little albumin. The specific gravity of the urine generally shows a marked fall on the 5th day. The percentage of chlorides at first increases and later diminishes as the kidneys become less efficient.

4. The weight of the adrenal glands is increased by one-third. Autolytic changes occur, chiefly in the medullary cells.

5. Kymographic observations show that there is a diminution in the pressor effect of adrenal gland extracts during the 48 hours following the subdural inoculation, that a recovery takes place and that towards the end of the disease there is a second diminution in the quantity of pressor substance.

6. Chemical tests agree in demonstrating a diminution of adrenalin in the adrenal glands in rabbits succumbing to fixed-virus rabies. Chemical tests also show a diminution of adrenalin in guinea-pigs dying from intramuscular inoculation with street-virus.

7. The cells of the secreting tubules of the kidneys are seriously damaged during the course of the disease.

8. The pancreas, liver and spleen seem to be scarcely damaged at all.

## CONCLUSION.

Owing to the growth of the rabies organism in the central nervous system, irritative stimuli pass along the splanchnic nerves to the adrenals and liver and give rise to the discharge into the blood stream of an excess of sugar. There may or may not be an accompanying excessive secretion of adrenalin.

Much damage is caused both to the kidneys and to the adrenals by toxic agents in the blood derived from the growth of the rabies organism in the central nervous system.

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- |                               |   |
|-------------------------------|---|
| 1. BULCHE and WEIS ..         | .. Deut. Arch. Klin. Med., 123, 1917, p. 163. |
| 2. FOLIN, CANNON and DENIS .. | .. Journ. Biol. Chem., XIII, 1912-13, p. 477  |
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# THE PHARMACO-DYNAMICS OF QUININE.

## II. SOME EFFECTS OF QUININE ON THE KIDNEYS, ADRENALS AND SPLEEN OF HEALTHY RABBITS.

BY

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[Received for publication, May 30, 1919.]

THE object of this investigation was to find whether quinine administered to healthy animals for a long period has any deleterious effect on the kidneys, adrenals and spleen.

Quinine is regularly taken by large numbers of persons in the tropics whose vocations lie in malarious parts. Does this dosage have any permanently injurious effect on them and, if so, is it better to accept this injury rather than to run the risk of an occasional attack of malaria?

There is no need to discuss here the value of quinine as a prophylactic agent or the principles which should guide one in its administration for this purpose. The point is that there are many persons in the tropics who use quinine habitually, and even dose their small children almost daily, with a sort of vague idea that they will get fever if they discontinue the quinine, whereas in fact they may be incurring little or no risk of infection with malaria from the nature of their surroundings. Can any of the impaired health which is attributed to residence in the tropics be fairly laid at the door of injudicious, and perhaps unnecessary, dosage with quinine? A complete survey of the effects of long continued dosage with quinine has yet to be made: the present paper merely

offers evidence that quinine is not without permanent effect on the healthy organism and is not a final answer to the queries put above.

#### DETAILS OF EXPERIMENT.

Three healthy young rabbits were chosen. As there was difficulty in giving them quinine orally, the intravenous route was used and latterly the intramuscular route. Quinine acid hydrobromide was used throughout, in solutions of the strengths at first of 1 in 60 and latterly of 1 in 30. Only one dose was given on any one day.

TABLE I.

*Showing quantity of quinine given.*

Month.	Number of days on which doses were given.	Total weight of the quinine salt given.	Route.
1918 July .. ..	7 days	168 gram	Intravenously.
„ August .. ..	17 „	544 „	„
„ September .. ..	4 „	128 „	„
„ October .. ..	4 „	128 „	„
„ November .. ..	nil	nil „	
„ December .. ..	8 „	296 „	„
1919 January .. ..	3 „	120 „	„
„ „ .. ..	19 „	1015 „	Intramuscularly.
„ February .. ..	20 „	1999 „	„
„ March .. ..	9 „	799 „	„
		5197 grams total.	

All three rabbits went through the course of treatment shown above. Rabbit 1 was killed on March 26th and its organs sectioned.

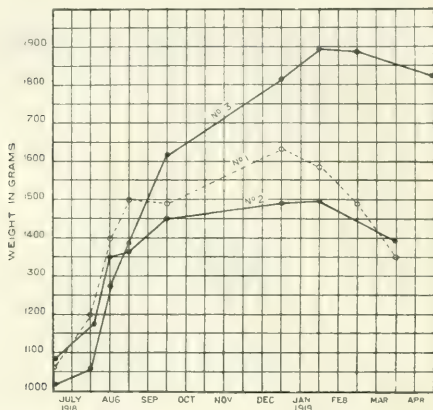
Rabbit 2 had a few more doses, or 5363 grams in all, before it was killed on March 31st.

Rabbit 3 had a few more doses both intravenous and intramuscular, or 6061 grams in all, and was killed on April 20th.

The intramuscular route was used only when the auricular veins had ceased to be pervious.

The Chart shows the weight of the rabbits at different periods of the quinine treatment. The more severe intramuscular dosage had a decided influence on the weight curves.

CHART.



Let us suppose that the average weight of these three rabbits throughout the experiment was 1,500 grams. Then 1,500 grams of rabbit had 5.197 grams of quinine acid hydrobromide in  $8\frac{1}{2}$  months, or about 255 days. This works out to 0.02 gram daily, or to 0.013 gram daily per kilo of rabbit.

If a man of 130 lb., or 59 kilos, were to take 5 grains of quinine daily for 255 days, the quantity would amount to 85 grams in all, or to 0.333 gram daily; in other words, 0.0056 gram daily per kilo body weight. Ten grains a day would amount to 0.0112 gram daily per kilo, which is a little less than the rabbits had.

Most quinine eaters, however, use either the sulphate or the hydrochloride, which both contain a higher proportion of the alkaloid than the hydrobromide contains.

A man of 59 kilos, taking 10 grains of the sulphate daily, would swallow, therefore, a weight of alkaloid equal to that found in 0.0147 gram quinine acid hydrobromide, while a man who happened to use the hydrochloride would swallow a weight of alkaloid equal to that found in 0.0152 gram of the acid hydrobromide.

It follows that the dosage administered to the rabbits was equivalent to something less than ten grains of quinine sulphate administered daily to a man of 130 lb. weight for a period of  $8\frac{1}{2}$  months, but in a somewhat irregular manner, days being missed and the dose being sometimes smaller and sometimes larger than 10 grains.

## THE ADRENALS.

TABLE II.

Animals.	Mean weight of adrenals.	Mean weight of adrenals per kilo body weight.
Normal rabbits .. .. .	0.223 gram	0.21 gram.
Three rabbits treated with quinine ..	0.510 "	0.33 "

TABLE III.

Animals	Grams adrenalin per gram of gland.	Grams adrenalin per kilo body weight.
Normal rabbits .. .. .	0.001595	0.000301
Three rabbits treated with quinine ..	0.000678	0.000265

The estimation of the quantity of adrenalin present was made by the method of Folin, Cannon and Denis(1). It appears from an inspection of Tables II and III that there is an enlargement of the adrenal glands in rabbits which have been treated with quinine for a long period. There is, however, no corresponding increase in the total quantity of adrenalin contained in the glands: it remains about normal or is a trifle reduced.

In this connection it is interesting to note that Ramsden, Lipkin and Whitley(2) found that after intraperitoneal injections in guinea-pigs the adrenals take up quinine at much higher concentration than any other tissue examined, and that the kidneys probably come next in the series. One might expect both these organs, therefore, to exhibit histo-pathological changes as a result of the fixation of quinine by their component cells, apart from any irritative effect that might accompany the excretion of the drug or any of its metabolites.

## MICROSCOPICAL EXAMINATION OF SECTIONS OF THE ADRENAL.

There were slight changes in the nuclei and cytoplasm of the cells of the medulla. In the cortex the usually well defined outline of the cells was lost in some regions and the nuclei were deeply stained and altered.

In Rabbit 3, the medullary cells were seemingly healthy, but the medulla as a whole was very small in size. In the cortex there were scattered necrotic areas, chiefly in the more central parts, due perhaps to thrombosis in terminal arterioles (*vide* photomicrograph 4). Many other areas covered by only two or three cells showed the beginning of necrotic changes. Quinine certainly has a noxious effect on the adrenals leading to enlargement and cellular degeneration most marked in the cortical region. How far their function may be deranged remains a matter for speculation.

It was unfortunate that the adrenals of these animals were required for two purposes, microscopical examination and the chemical estimation of the adrenalin. Neither examination could, therefore, be complete, so it would be unwise to lay too much stress on the findings reported above.

## THE KIDNEYS.

TABLE IV.

Animals.	Mean weight of kidneys.	Mean weight of kidneys per kilo body weight.
Normal rabbits .. .. .	8.14 grams	7.52 grams.
Three rabbits treated with quinine ..	10.66 „	6.90 „

If there is any change it is on the side of a slight diminution in weight.

## MICROSCOPICAL EXAMINATION OF KIDNEYS.

*Rabbit 1.*—

Granular and cellular plugs in many of the collecting tubules (*vide* photomicrograph 1).

Cellular degeneration and epithelial denudation in many of the secreting tubules.

Hæmorrhages into and obliteration of some glomerular tufts.

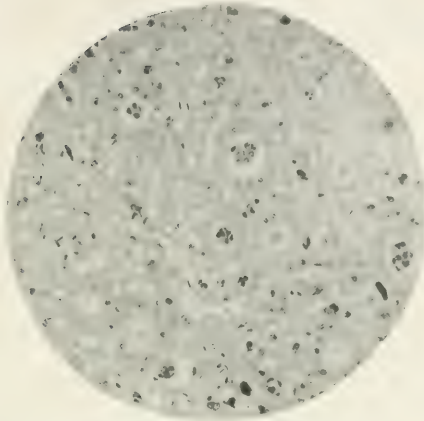
Some glomeruli changed into fibrous nodules.

A few fibrotic patches occluding and replacing tubules.



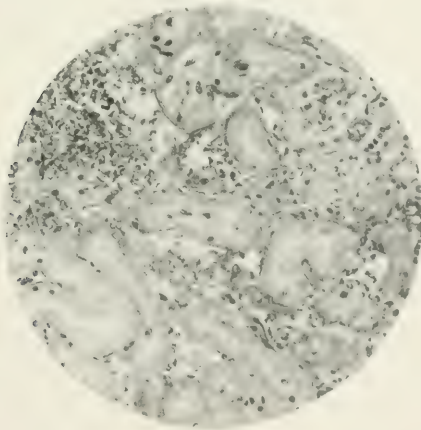
PLATE IV.

PHOTOMICROGRAPH 1.



Collecting tubules of kidney of a normal rabbit treated with quinine, showing many tubules blocked by plugs of epithelial cells.

PHOTOMICROGRAPH 2.



Secreting tubules of kidney of a normal rabbit treated with quinine, showing hæmorrhage round the tubules and destruction of their secreting epithelium.

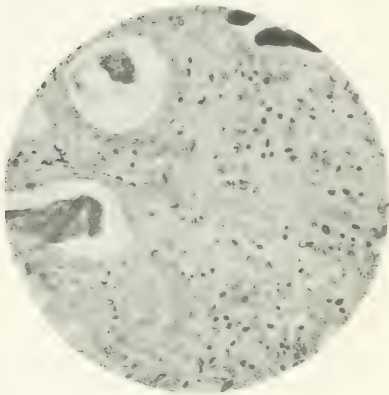
J. W. CORNWALL.—The Pharmacodynamics of Quinine.





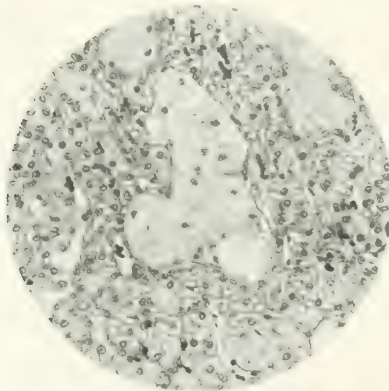
PLATE V.

PHOTOMICROGRAPH 3.



Kidney of a normal rabbit treated with quinine, showing small round cell infiltration proceeding to fibrosis and contraction. The remains of secreting tubules can be seen with their epithelial lining almost destroyed. Other tubules have been blocked lower down leading to accumulations of fluid secretion in their upper parts which have formed cysts.

PHOTOMICROGRAPH 4.



Cortex of adrenal of a normal rabbit treated with quinine, showing necrotic areas presumably due to thrombosis in terminal arterioles.

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Some fairly extensive hæmorrhages round tubules in the glomerular region (*vide* photomicrograph 2).

*Rabbit 2.*—

Epithelial and granular plugs in some collecting tubules.

Some minute hæmorrhages.

Epithelial exfoliation in some secreting tubules.

Secreting epithelium tumid where exfoliation is not occurring.

*Rabbit 3.*—

Epithelial plugs in some collecting tubules.

Much degeneration of tubular epithelium, lumina very wide.

Thickening of Bowman's capsule and progressing obliteration of some glomeruli.

Small round cell infiltration beginning here and there, with fibrous contraction leading to obliteration of some glomeruli and tubules.

Extension of fibrous bands from capsule obliterating many secreting tubules near the surface and giving rise to the formation of small cysts (*vide* photomicrograph 3).

The kidneys of all three rabbits showed a state of sub-acute inflammation progressing towards fibroid changes with obliteration of some of their secreting elements. The changes in all three were similar, being least advanced in No. 2 and most advanced in No. 3.

Quinine has a noxious effect on the secreting epithelium of the kidneys. Some of the tubules and glomeruli were permanently effaced and the functioning power of the organ was proportionately reduced in the experimental animals.

# THE SPLEEN.

TABLE V.

Animals.	Mean weight of spleen.	Mean weight of spleen per kilo body weight.
Normal rabbits .. .. .	0.42 gram	0.38 gram
Three rabbits treated with quinine ..	0.46 „	0.29 „

The spleen was reduced in weight about 20 per cent in the rabbits treated with quinine.

Microscopically there was not much deviation from the normal, except that numerous small masses of blood pigment were seen, especially in rabbit 3. This was being actively taken up by phagocytes. The indication is that destruction of red corpuscles was proceeding with greater rapidity than in healthy animals.

#### SUMMARY.

There is evidence that quinine administered to healthy rabbits intravenously and intramuscularly over a period of 8-9 months causes damage to the cellular elements of the adrenals and of the kidneys. There is also evidence that the rate of disintegration of red blood corpuscles in the spleen is increased.

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2. RAMSDEN, LIPKIN and  
   WHITLEY     *Annals of Tropical Medicine and Parasitology*, XI,  
                  1918, p. 223.

# THE PATHOGENESIS OF DEFICIENCY DISEASE.

## No. III. THE INFLUENCE OF DIETARIES DEFICIENT IN ACCESSORY FOOD FACTORS ON THE INTESTINE.

BY

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[Received for publication, June 1, 1919.]

THE pathological changes in the intestine which result from dietaries deficient in accessory food factors have been studied in :

- (1) pigeons fed exclusively on autoclaved milled rice : that is to say, on food lacking in all three classes of accessory food factors ;
- (2) pigeons fed on autoclaved rice together with fresh butter and onions : that is to say, on food deficient in accessory food factors of the ' B ' class ;
- (3) pigeons fed on autoclaved rice together with fresh butter : that is to say, on food deficient in accessory food factors of the ' C ' class (anti-scorbutic substances), as well as in factors of the ' B ' class (the so-called ' anti-neuritic vitamines ' ) ;
- (4) guinea-pigs fed on a diet of crushed oats and autoclaved milk : that is to say, on food deficient in anti-scorbutic substances; and



- (5) guinea-pigs fed on an exclusive diet of autoclaved rice : that is to say, on food deficient in all three classes of accessory food factors.

While the morbid changes resulting from these dietaries are attributable in the main to deficiency of accessory food factors, their genesis is no doubt contributed to, or their effects enhanced, by deficiency of other essential attributes of a satisfactorily balanced ration.

All experiments were carried out under conditions and precautions previously detailed(1) : they were continued to a point at which death of the animals occurred or was about to occur.

#### I. THE EFFECTS OF AN AUTOCLAVED RICE DIETARY ON THE INTESTINE OF PIGEONS.

The pathological changes were studied macroscopically in 152 pigeons fed exclusively on milled or on autoclaved milled rice, and microscopically in 24. All these birds developed *polyneuritis gallinarum* in consequence of the deficient dietary.

##### *A.—Macroscopical Appearances.*

The chief macroscopical features observed at autopsy in the intestine were atrophy and congestion, both of which might be extreme. All cases presented atrophy in greater or lesser degree, while a proportion, estimated at about 30 per cent, showed no congestion appreciable by the naked eye.

The upper portion of the intestine of healthy pigeons, especially that part embracing the pancreas, is thick and muscular (Fig. 6). It is this portion which suffers most in consequence of the deficient dietary. The atrophy is of all grades of severity ; it may be comparatively slight, or progress to a point at which the intestinal walls are so thin as to be transparent. As a rule the atrophy is more marked the longer the bird is under the influence of the defective diet.

The congestion is commonly confined to the upper part of the alimentary tract ; less often it involves its whole extent. The branches of the mesenteric vessels surrounding the bowel may be greatly engorged, and extravasations of blood are occasionally to be seen under its serous coat.



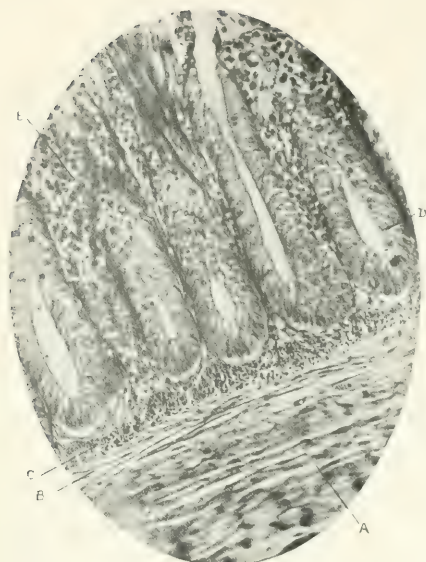


Fig. 1.



Fig. 2.



Fig. 3.

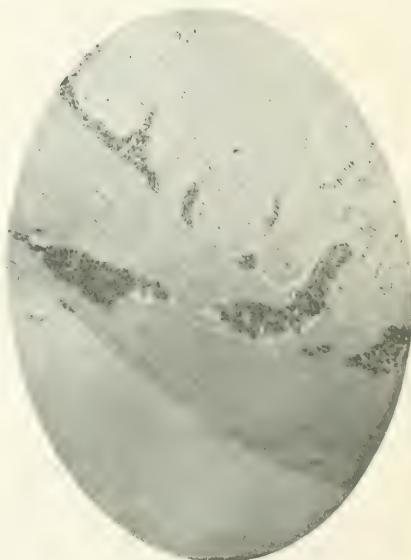


Fig. 4.

An attempt was made to correlate the degree of congestion with the degree of enlargement of the adrenal glands: no correlation could be established.

On splitting open the bowel, the upper three to six inches are often found to be distended with tapeworms, more rarely with round worms—the latter usually dead, the former almost invariably alive. Round worms have occasionally been found in the œsophagus.

On examining the surface of the bowel with the hand-lens, varying degrees of atrophy and erosion of the mucous membrane are encountered. These are not always uniformly distributed over the whole surface of the bowel; some areas show them in greater degree than others. Ecchymoses are common over the mucous surface; in the more severe cases pin-point hæmorrhages into the bowel lumen are to be seen.

### *B.—Histo-Pathology.*

Apart from some degree of atrophy of all coats of the bowel, which is universal in birds dying in consequence of this dietary, no marked histological changes, with the exception, it may be, of slight congestion, are found in approximately 30 per cent of cases. In the remainder the histo-pathological appearances are very striking, although widely variable in degree in different birds.

The pathological changes comprised the following:—

- (1) congestion and hæmorrhage;
- (2) atrophy of the myenteron;
- (3) degenerative changes in the myenteric plexus of Auerbach;
- (4) atrophic and inflammatory changes in the mucous membrane;
- (5) atrophy of lymphoid structures;
- (6) fibrosis; and
- (7) changes favouring systemic infection from the diseased bowel.

*Congestion and hæmorrhage.*—In its mildest form, this change consists in engorgement of the subperitoneal vessels, and in distension of the small vessels ramifying between the muscularis mucosæ and the base of the crypts of Lieberkuhn, or the glandular structures corresponding to these in birds (Figs. 1 and 2). The process of congestion is not usually limited to distension of vessels in these areas, but may extend to those of the villi. The next stage in the congestive process is the occurrence of actual hæmorrhages around the bases of the crypts (Figs. 2, 4, 5). In some cases an almost continuous layer of hæmorrhage may surround the

bowel at this point (Figs. 4, 5). Such remnants of villi as remain are often seen to be much engorged or to contain effusions of blood: this appearance is frequently very pronounced in the lower bowel (Figs. 10, 11). Free corpuscles may occasionally be found in the lumen of the bowel or exuding from its congested and frayed mucous membrane.

A further, or simultaneous, stage in the process is the spread of these hæmorrhagic areas from the submucous coat into the myenteron: these may involve only the circular layer of muscle fibres or penetrate to the longitudinal layer. Illustrations of such are shown in Figs. 4, 5: these represent hæmorrhages of moderate extent. It is not unusual to see larger tracts of the circular layer of muscle broken up by extensive hæmorrhages into its substance. Such hæmorrhages cause separation and rupture of the muscle fibres. The ruptured fibres may present ragged edges enclosing the collection of effused blood or, as in Fig. 3, the hæmorrhages may be more diffuse, the corpuscles being scattered throughout the whole thickness of the myenteron and penetrating to the serous coat.

Added to these appearances is the engorgement—often intense—of the vessels running under the serous coat and ramifying in the muscular layers. Oblique and longitudinal sections of these vessels may at first sight present the appearance of hæmorrhagic effusions. Rupture of the vessel walls is, however, rare.

Fig. 5 shows an uninterrupted stream of blood corpuscles extending from an engorged and atrophic villus—the epithelial covering of which is incomplete—directly into the large subperitoneal vessels.

Œdematous infiltration of the coats of the bowel has occasionally been encountered.

Intense congestion of, and hæmorrhages into, the coats of the bowel, —and especially into the coats of the upper bowel—sometimes leading to actual breaches in their continuity, are, therefore, amongst the results of a dietary deficient in all classes of accessory food factors. It may be assumed that resolution of such hæmorrhages would lead to the deposition of fibrous tissue in the myenteron and serous coat.

*Atrophy of the myenteron.*—The average thickness of the circular layer of the muscular coat, at a point one to two inches from the stomach, is shown in Fig. 6. It is not of the same thickness at every point of the bowel's circumference, but gradually merges from a thickness of about 0·35 m.m. to one of about 0·25 m.m.

In pigeons fed on the deficient diet the degree of atrophy of the circular muscular layer varies considerably. The muscular coat is usually thinnest in birds which survive longest, or in which infection of the bowel walls has occurred; it is atrophied, as a rule, to the extent of one half to one quarter its normal thickness in health (Figs. 7, 8, 9), and its efficiency is thus proportionately reduced. Added to this are frequent hæmorrhages, the rupture and separation of muscle fibres thereby, and, less commonly, segmentation and fragmentation of these fibres (Fig. 3). The extent to which the motor function of the bowel may be impaired by the deficient dietary will be realized from these statements.

*Degenerative changes in the myenteric plexus of Auerbach.*—Careful scrutiny is necessary in order to detect, in healthy birds, the ganglionic swellings of this plexus, as they lie between the circular and longitudinal layers of the myenteron. They are compact bodies of oval form varying considerably in size, the variations being of course dependent on their point of section. They fit snugly between the layers of muscle and give rise to little or no bulging in the contour of these layers.

In hæmatoxylin-stained sections of the intestine from birds fed on the deficient dietary these ganglia are much more obvious and catch the eye at once. Their readier recognition may be due in part to the fact that the atrophy and recession of the circular and longitudinal muscle fibres renders them more prominent. Their greater prominence is, however, often the result of swelling, due probably to œdematous infiltration. In size they vary within wide limits, dependent in some measure on their point of section. In general, they are double or treble the size of healthy ganglia.

The swollen ganglia have frequently been noted to cause bulgings of the longitudinal muscular layer towards the serous covering of the bowel or of the circular layer towards its lumen, or both (Fig. 12). The cytoplasm of the ganglionic cells may appear shrunken, their nuclei more excentric than normal, and the nucleoli almost invariably fragmented, the last appearance being the most constant. Occasionally, whole cells or groups of cells appear to have disappeared, leaving in the ganglion lacunæ empty of cellular structure.

There can, I think, be little doubt that the plexus myentericus is involved in a degenerative process. This being so, it may be concluded that the nervous control of the bowel is impaired in proportion to the degree of degenerative change.



*Atrophic and inflammatory changes in the mucous membrane.*—These consist in (a) atrophy and partial or complete disappearance of the villi; (b) frequent congestion of such villi as remain; (c) atrophy of the glandular cells of the crypts of Lieberkuhn, and separation of these from their basement membrane; (d) partial or complete disappearance of the lymphoid cells lying between and around the crypts; (e) thinning, or actual disappearance in places, of the muscularis mucosæ; (f) a relatively greater proportion of reticular cells, and the scattered deposition of fibroblasts; (g) an increased proportion of leucocytes in the atrophic mucous membrane, and, more rarely, (h) bacterial invasion with intense inflammatory and necrotic changes in the mucous membrane and underlying coats of the bowel.

These appearances are well shown in Figs. 3, 7, 8, 9, 12, and call for little further description. They are, as a rule, most marked in birds which have survived the dietary for the longest time, or in which infections of the bowel wall are found at autopsy. They are rarely present in the same degree at every area of the bowel's circumference. Sections of even the most severely affected intestines may show some areas in which these changes are much less marked than in others.

*Atrophy of lymphoid structures.*—In the healthy pigeon the lymphoid cells fill in the spaces between the crypts, and present a continuous layer of varying thickness (Fig. 1) between the bowel lumen and the muscularis mucosæ. In diseased birds this protective layer is atrophied in greater or lesser degree. In some areas it was completely wanting (compare Figs. 1 and 3); and the muscularis mucosæ also had frequently disappeared.

*Fibrosis.*—The evidences of fibrosis are scanty in so far as the mucous membrane itself is concerned. The loss of lymphoid cells and the atrophy of the secretory elements bring into greater prominence the fibrous reticulum (Fig. 3). Areas, however, are encountered in which there is a deposition of scattered fibroblasts.

Some long-standing cases present areas of fibrotic infiltration in the muscular coats; but this being a reparative process, it is likely to be encountered only rarely in birds not permitted to recover. It may be expected that where such recovery is brought about the resolution of the hæmorrhagic effusions will give rise to fibrotic deposits in the muscular and serous coats of the intestine.

*Infection from the diseased bowel.*—Systemic infection is rendered more easy in the presence of these pathological processes owing (1) to



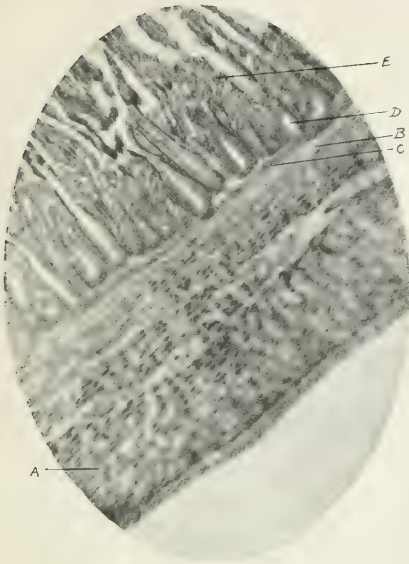


Fig. 6.



Fig. 7.



Fig. 8.



Fig. 9.



11  
12

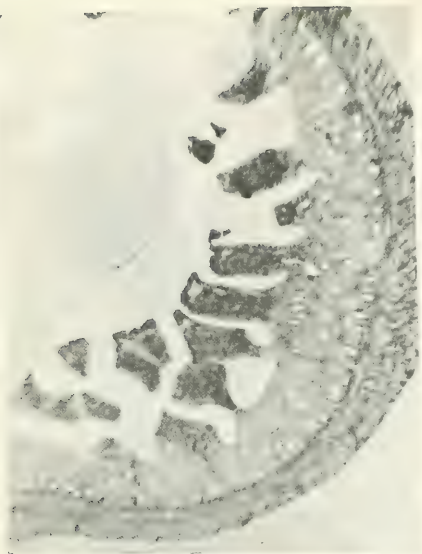


Fig. 10.

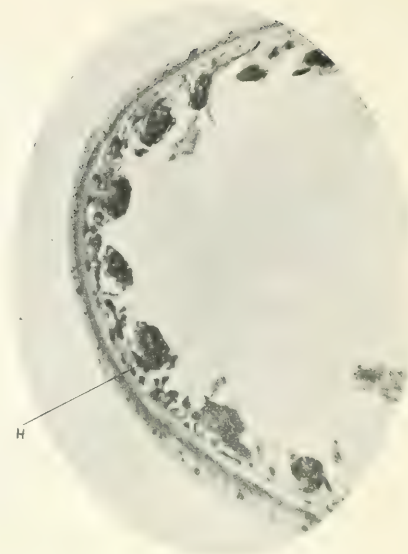


Fig. 11.



Fig. 5

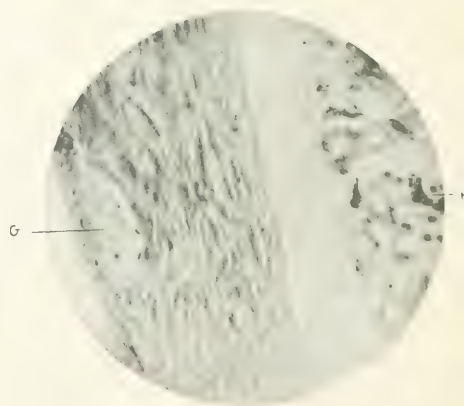


Fig. 12.

impaired production of digestive juices; (2) to continued congestion of the mucous membrane and consequent malnutrition of its secretory cells; (3) to loss of the protective layer of lymphoid cells; (4) to increased leucocytic invasion of the mucous membrane and to increased leucocytic traffic in micro-organisms between the bowel mucous membrane and the blood stream; (5) to the greater facilities which the debilitated mucous membrane provides for the growth of bacteria on its surface and in its substance; (6) to the imperfect digestion of food in the upper alimentary tract, which affords a favourable medium for the growth of micro-organisms; and, although more rarely, (7) to actual breaches of continuity in the walls of the bowel itself. Occasional specimens have shown a direct continuity of blood corpuscles from an eroded villus to the blood vessels running external to the serous coat (Fig. 5).

Cases are encountered with comparative frequency in which the atrophic and congestive processes had superadded to them the effects of intense bacterial invasion of the bowel walls. In such cases bacteria of various types were to be found in large numbers throughout the necrotic mucous membrane (Figs. 7, 8, 9, 11) and lying also in the submucous and muscular coats. By special staining methods bacteria (chiefly cocci) have been detected in the vessels of the submucosa, and bacteria-laden leucocytes are common objects in the microscopic field. In these circumstances, bacterial organisms may pass directly into the blood-stream from the infected bowel walls. In four such cases I cultured from the heart's blood at autopsy, by aerobic methods, a small coccus in two, a coliform organism in a third, and bipolar organism in a fourth. Infection of the blood from the bowel is therefore no uncommon consequence of "vitaminic" deficiency.

Figs. 6, 7, 8, 9 represent a series of sections of the bowel of the same magnification. In these the effects of the atrophic, congestive and necrotic processes, found in cases where intense infection of the bowel walls has occurred, are well seen. The mucous membrane may be so destroyed and the changes in the bowel walls so pronounced as to render recovery impossible. One is occasionally surprised to find that, while in one case the administration of 'vitaminic' extracts, or of whole grains of *mung dal* (a species of small pea), will cure 'polyneuritis' with surprising rapidity, another, to all appearances identical with it, will succumb. Amongst the reasons for this are the varying degrees of pathological change and of bacterial invasion of the bowel walls as well as systemic infection therefrom (*vide infra*). It is evident that the ingestion of

specific pathogenic organisms (such as *B. dysentericus*, *B. typhosus*, *E. histolytica*, etc.) in the presence of 'vitaminic' deficiency is likely to be followed by their implantation on the debilitated bowel mucous membrane.

In connexion with these lesions in the upper alimentary tract of pigeons fed exclusively on autoclaved milled rice it is to be remembered that Hamilton Wright(3) was so impressed with the constancy of pathological changes in this situation in human beri-beri that he attributed this malady to a specific gastro-duodenitis. As is now well known, the pathological changes he described are not the cause of beri-beri; they are, however, important evidences of deficiency of certain accessory food factors in the diet. The fact that Hamilton Wright found a bacillus of constant morphological character in this situation is important. Although the organism is not the specific cause of beri-beri, its presence is an illustration of the fact that the wall of the upper intestine may become infected in the human subject in consequence of such a dietary.

## II. THE EFFECTS OF A DIETARY OF AUTOCLAVED RICE, BUTTER AND ONIONS ON THE INTESTINE OF PIGEONS.

A dietary of autoclaved rice, butter and onions, while it is deficient in accessory food factors of the water-soluble 'B' class, contains an abundance of anti-scorbutic substances and of accessory food factors of the fat-soluble, or 'A' class. Forty-two pigeons were fed on this dietary: all developed *polyneuritis gallinarum*. The birds were noted to have eaten greedily of the chopped onions—sparingly of the rice. The fresh onions used must have contained but a scanty supply of accessory food factors of the 'B' class, since the onset of *polyneuritis gallinarum* was not prevented thereby although considerably delayed(4).

The macroscopical evidences of pathological change in the intestines of pigeons so fed were studied in forty-two cases. The microscopical changes were studied in serial sections of the upper, middle and lower bowel in twelve cases.

It was noted that naked-eye evidences of congestion, hamorrhage and other pathological processes were less frequently encountered in pigeons whose basal diet of autoclaved rice was enriched with fresh butter and fresh onions; notable evidences of congestion were slight or nil in 50 per cent of all cases.

The atrophy of the bowel, although often considerable, was usually less marked than in cases dying in consequence of complete 'vitaminic' starvation. Histologically one did not, as a rule, encounter the same degree of congestion: actual hæmorrhages were more rarely met with, while the degree of atrophy of the myenteron and of the elements of the mucous membrane, although usually considerable, were often comparatively slight. Two cases were encountered amongst twelve examined where bacterial infection of the bowel walls had assisted in bringing about changes as pronounced as those so commonly seen in the case of birds wholly deprived of accessory food factors.

Fresh butter and onions when added to a dietary of autoclaved rice thus provide some at least of those factors on which the functional perfection of the alimentary tract is dependent.

### III. THE EFFECTS OF A DIETARY OF AUTOCLAVED RICE AND BUTTER ON THE INTESTINE OF PIGEONS.

The effects of this dietary, which is deficient in 'vitamines' of the 'B' and 'C' classes and gives rise to typical *polyneuritis*, were studied macroscopically in eighteen cases and microscopically in six.

The description given of the effects of complete 'vitaminic' starvation applies to these cases. The butter used afforded the birds no protection in so far as the intestines were concerned. Figs. 6-9 typify the appearances seen.

With the reservation that the number of birds in this category is small in comparison with those in the two previous categories, one is led to suspect that, since butter did not protect, it was the addition of fresh onions to the basal diet of autoclaved rice that afforded the birds in the second category the measure of protection against congestive and hæmorrhagic processes which they undoubtedly enjoyed.

In order to provide further evidence on this point, the appearances presented by the intestines of guinea-pigs fed on dietaries (a) of crushed oats and autoclaved milk, and (b) of autoclaved rice, were studied.

### IV. THE EFFECTS OF A SCORBUTIC DIET ON THE INTESTINE OF GUINEA-PIGS.

Twelve guinea-pigs, with an equal number of controls, were employed in this experiment. The animals were isolated in separate cages. They



were fed on crushed oats and autoclaved milk. The oats were locally grown and were of poor quality. The milk was autoclaved at a temperature of 130°C. for one hour. The experiment was continued to the death of the pigs, which occurred within periods ranging from twenty to thirty days. Autopsies were performed immediately after death with the detail and precautions previously described(1). Three of twelve guinea-pigs were excluded owing to the presence of complicating pathological processes, such as peritonitis. The macroscopical appearances found in the gastro-intestinal tract were studied in nine cases; the microscopical in five.

#### *A.—Macroscopical Appearances.*

The most notable feature present was intense congestion of the duodenum. This part of the bowel, for a variable distance from the pylorus, was in four out of nine cases so congested as almost to present the appearances seen in strangulated bowel. The congested area was sharply demarcated by the pylorus and extended down the bowel for a distance of from one to two inches. In two other cases the congestion of the duodenum was of a patchy character. In the three remaining cases moderate congestion of the whole bowel was present in two, while no appreciable congestion was present in the third.

In contradistinction to the appearances presented by the upper intestine of pigeons, where the bowel is often greatly thinned, the duodenum of guinea-pigs was usually swollen and turgid, the tumefaction being due largely to hæmorrhagic infiltration of all coats of the bowel.

On opening the duodenum and examining the mucous surface with the hand-lens, ecchymoses were frequently to be seen, while areas resembling punched-out ulcers, whose base extended almost to the peritoneal coat, were occasionally encountered. At these areas the bowel walls were almost transparent.

The intestinal tract is thin in healthy guinea-pigs. Apart from the duodenum, the bowel walls in pigs dying in consequence of the deficient dietary were, generally speaking, thinner than in health. In three cases of the nine referred to, punched-out necrotic ulcers were found in the stomach. In one case as many as fifteen were counted, the base of the ulcer extending down to the peritoneal coat, the mucous membrane of the stomach being studded with the brownish debris of hæmorrhages into the interior of the organ. Thus localized, destructive changes in the



PLATE IX.

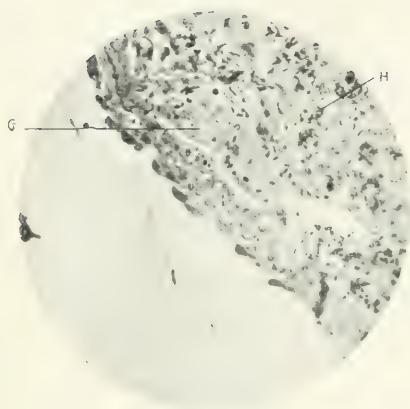


Fig. 13.

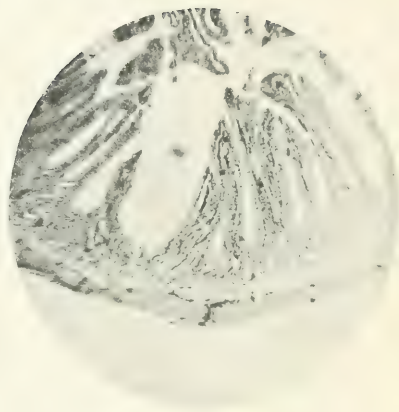


Fig. 14.

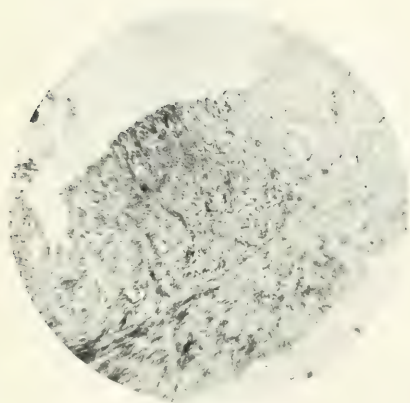


Fig. 15.



Fig. 16.

mucus and underlying coats of the stomach and duodenum are amongst the more occasional results in guinea-pigs of a dietary of crushed oats and autoclaved milk.

These changes were present in the gastro-intestinal tract of guinea-pigs, dying in consequence of this dietary, which exhibited none of the characteristic naked eye appearances of scurvy. In a clinical sense then they may be regarded as pre-scorbutic.

### *B.—Histo-Pathology.*

The microscopical appearances seen in sections of the duodenum in these cases comprised:—

- (1) turgidity, with diffuse hæmorrhagic infiltration of all coats of the bowel;
- (2) degenerative changes in the myenteron;
- (3) degenerative changes in the myenteric plexus; and
- (4) atrophic and necrotic changes in the cellular elements of the mucous membrane.

These lesions resemble closely those described in the case of pigeons fed on an exclusive dietary of autoclaved rice. In general the same description applies to both. In the case of guinea-pigs the more diffuse hæmorrhagic infiltration of the mucous membrane may impart to it a degree of turgidity which is not seen in pigeons (Figs. 14 and 15). The degenerative process involves the ganglia of the myenteric plexus in a manner similar to that described in pigeons. In some cases, more especially in the vicinity of extensive hæmorrhagic infiltration of the myenteron, the normal histological structure of the ganglia may be much altered. In these circumstances, ganglia may be encountered which are infiltrated with blood, and in which no single cell contains a normal nucleus. As in the case of diseased pigeons so also in guinea-pigs, the myenteric plexus is much more prominent than in health. In hæmatoxylin-stained sections the ganglia appear swollen, the nerve cells ill-defined, the nuclei degenerated, and the nucleolus fragmented. Not all cells, however, are so affected in either species, some exhibiting nuclei of apparently normal structure. Without definitely asserting that the degenerative changes in the myenteric plexus are more pronounced in guinea-pigs than in pigeons, they certainly appear to be no less. There can be little doubt that neural lesions (Fig. 13), which may impair the nervous control of the bowel, result in consequence of

this dietary ; further histological study by special methods is necessary to determine their character.

The myenteron also is often, although not always, greatly disorganized in consequence of diffuse hæmorrhagic infiltration of its substance ; in such circumstances separation of muscular fibres with their segmentation and fragmentation are common appearances (Figs. 19 and 20). The degenerative and necrotic changes in the mucous membrane are as pronounced in guinea-pigs as in pigeons, and are of similar character (compare Figs. 3, 18, 19, and 20). The same comments as to the facilities which these pathological processes afford for infection of the bowel walls and for systemic infection therefrom, apply with equal force to both species.

The histological changes in the lower bowel are of like character to those found in the upper bowel, but more moderate in degree.

The histo-pathological changes found in guinea-pigs are illustrated in the series of photo-micrographs numbered 13 to 20. If these are contrasted with Figs. 1 to 12, which illustrate the histo-pathological changes found in Aves, their similarity will become very apparent.

#### V. THE EFFECTS OF AN AUTOCLAVED RICE DIETARY ON THE INTESTINE OF GUINEA-PIGS.

For the purpose of this study, four guinea-pigs were employed. They were fed on an exclusive diet of autoclaved rice until death occurred, which event took place within 46 days of the initiation of the experiment.

My *post-mortem* notes with regard to the intestinal tract in these animals read as follows :—

No. 1. The duodenum shows intense congestion, the first inch-and-a-half looks almost gangrenous, so intense is the congestive process ; there is a well-marked ulcer at one point, its base extending to the peritoneal coat, so that only the serous covering appears to intervene between the bowel lumen and the abdominal cavity. Both small and large bowel are congested, while they appear thinner than in health.

No. 2. Perhaps the most interesting pathological appearance is that presented by the duodenum. This part of the bowel is much congested, its vessels being greatly engorged ; two hæmorrhagic areas of

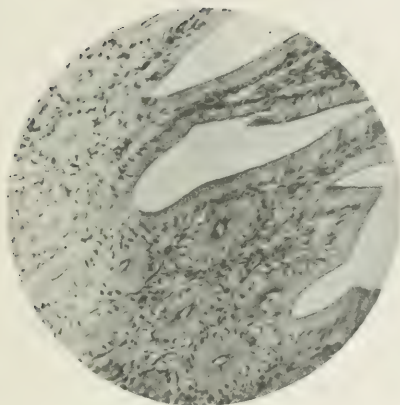


Fig. 17.

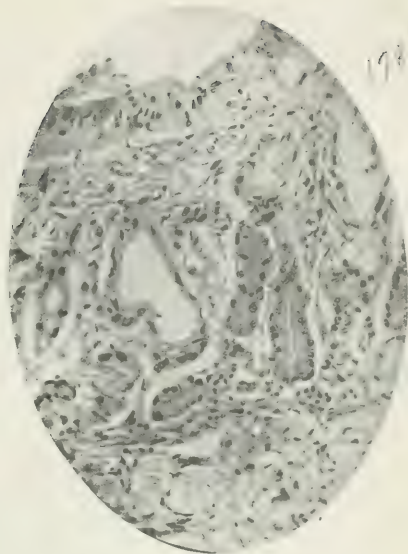


Fig. 18.

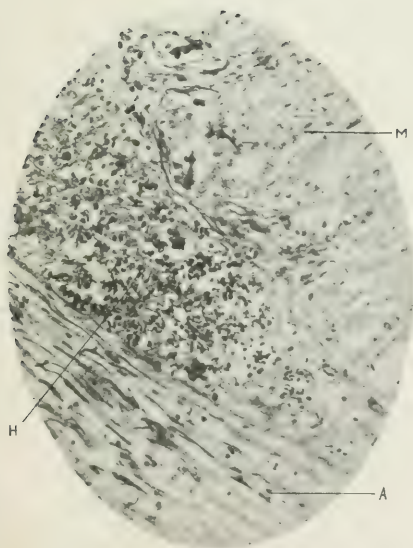


Fig. 19.

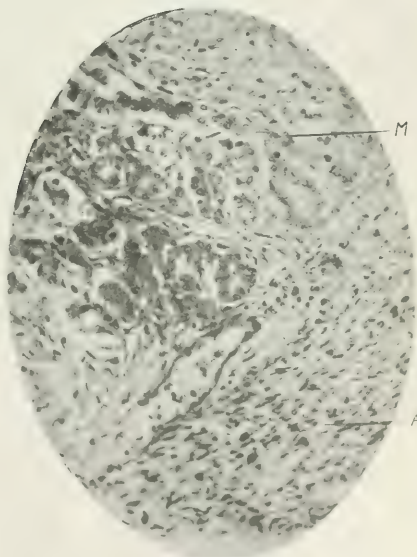


Fig. 20.





extensive proportions almost surround the bowel circumference. On opening the duodenum, congestion of the mucous membrane was seen to be pronounced and numerous ecchymoses were scattered over its surface; the viscus was transparent at one part, the peritoneal coat alone appearing to intervene at this area between the bowel lumen and the abdominal cavity.

No. 3. The duodenum is much congested and turgid and its mucous membrane is studded with capillary hæmorrhages. The rest of the bowel is excessively thin.

No. 4. Apart from a moderate degree of generalized congestion of the bowels, and abnormal tenuity, no noteworthy macroscopical characters were recorded.

It appears then that congestive, hæmorrhagic, atrophic and necrotic changes in the bowel walls, usually most pronounced in the upper intestine, are common consequences in guinea-pigs of dietaries deficient in accessory food factors. It will have been noted that in both Aves and in guinea-pigs, the pathological processes are similar in character as well as in distribution. The relatively less frequent presence of these lesions in pigeons whose dietary was deficient in the 'B' factor only, the fact that pigeons deprived of both 'B' and 'C' factors suffered from pathological lesions of the bowel as pronounced as did those deprived of all three classes of accessory food factors, the fact that these lesions were equally well-marked in guinea-pigs subjected to complete 'vitaminic' starvation and in guinea-pigs deprived only of the 'C' factor, indicate that the congestive and hæmorrhagic lesions in the intestines of both species are largely, if not mainly, due to the absence of fresh green foods from the dietary. Control guinea-pigs fed on crushed oats, autoclaved milk and on abundance of fresh vegetables were wholly protected from gastro-intestinal lesions. It is worthy of comment that the functional perfection of the gastro-intestinal tract in herbivorous animals, appears to be dependent in considerable measure on accessory food factors found in fresh vegetable foods.

Having regard to the readiness with which accessory food factors of the 'C' class are destroyed in the process of cooking, their absence from the dietaries of human beings, or their long-continued sub-minimal supply, may have an important bearing on the genesis of gastro-intestinal disorders. These observations appear to provide

an explanation of the beneficial action of orange juice on the digestive and assimilative processes in bottle-fed infants.

Before leaving this subject, I would again direct attention to the fact that the intestinal lesions I have described in guinea-pigs were present in the majority of my cases before clinical evidences of scurvy were apparent. I suggest, therefore, that the symptoms of scurvy, as described in text-books, are the gross evidences of a disordered state of metabolism, the minor or pre-scorbutic manifestations of which are probably frequently overlooked, especially in children.

#### COMMENTARY.

Interpreted in terms of bowel function, the derangements to which such pathological changes as have been described may ultimately lead can be grouped as follows:—

- (1) Impairment of the neuro-muscular control of the bowel; impaired transport of the intestinal contents along the alimentary canal.
- (2) Impairment of assimilative power.
- (3) Impairment of secretory function.
- (4) Impaired protective resources leading to infection of the mucous membrane of the bowel by pathogenic saprophytes or ingested bacteria, and to systemic infection therefrom.

Unwilling as I am to apply too directly to the human subject the experimental results of intensive 'vitaminic' starvation as observed in birds and in guinea-pigs, we may, I think, regard the changes I have described as applicable in some degree to man.

It is my experience that, while complete deprivation of accessory food factors will cause the relatively rapid appearance of so-called 'polyneuritis'\* in birds, sub-minimal provision of these substances, protracted over prolonged periods of time, will, especially in young and growing birds, eventually lead to a like morbid state. It is, I think, with sustained sub-minimal supply of accessory food factors that the physician is mainly

\* The term 'polyneuritis', as applied to the morbid state resulting in pigeons from an exclusive dietary of autoclaved milled rice, is a misnomer. It suggests that the nervous tissues are exclusively or mainly involved in the pathological process, whereas in reality no organ or tissue escapes the consequences of 'vitaminic' deficiency; some, indeed, such as the reproductive organs, the spleen, the thymus, and the digestive organs, are more gravely affected than is the nervous system.—R. McC.

concerned in practice. Complete 'vitaminic' deprivation is, as I have pointed out in another place, relatively rare<sup>(1)</sup>; incomplete "vitaminic" deprivation—or sub-minimal supply of accessory food factors—is relatively frequent. If, in the light of the histo-pathological findings here recorded, we consider the case of children fed, it may be from birth, on foods deficient in these essential substances, and the frequency with which in later life their dietary continues to be ill-balanced and defective, we are, I venture to think, in a position to realize the ultimate consequences of such a dietary and to anticipate the sequence of events leading up to grave derangements of bowel function. Food deficiency also prepares the soil for bacterial growth and the resultant morbid states will vary with the nature of the organisms which may become implanted upon it. This knowledge, if it does not enable us to account fully for such gastro-intestinal disorders as mucous disease, coeliac disease, and chronic intestinal stasis, will at least help us to a better understanding of their genesis.

#### I. MUCOUS DISEASE.

I have referred to the fact that pigeons fed on an exclusive dietary of autoclaved rice frequently suffer from diarrhoea<sup>(4)</sup>, and have pointed out that this symptom of 'vitaminic' deficiency can, in a proportion of cases, be made to disappear, often with surprising rapidity, by the administration of the alcoholic extracts of the yolks of eggs. Others have drawn attention to the almost constant occurrence of diarrhoea or of colitis as precursors or concomitants of the condition known as 'war oedema,' a malady proved to be due to food deficiency<sup>(5)</sup>. Clinical evidence of bowel derangements due to this cause are, therefore, not lacking. If, then, we attempt, from the results of our histo-pathological observations, to provide an explanation of the origin of mucous disease, we may, I think, surmise that continued sub-minimal 'vitaminic' supply will lead to that state of chronic gastro-intestinal catarrh which characterizes this disorder.

Mucous disease is very common amongst European children in India who are fed largely on sterilized milk (milk, which, in my own experience, is often poor in 'vitaminic' content), artificial foods, white bread, polished rice, poor butter, over-cooked vegetables and excessive quantities of sugar. Therapeutic experience of this malady shows how readily it yields to limitation of carbohydrates and to a rationally-

balanced dietary of good 'vitaminic' quality. The mucous stools, the diarrhoea, the peevishness, the unhealthy appetite rapidly disappear on such a regimen, as also do the 'night terrors' from which the subjects of mucous disease so frequently suffer.

I do not wish to dogmatize with regard to the genesis of this malady. I would but draw attention to the fact that an ill-balanced diet, deficient in accessory food factors, is capable of giving rise in pigeons to a state of chronic congestion of the intestinal mucosa, often accompanied with hypertrophy of the adrenal glands; the relief afforded to children suffering from mucous disease by the correction of their ill-balanced dietaries may find an explanation in this fact.

## II. CÆLIAC DISEASE.

One cannot read the illuminating account of this disorder given by Dr. G. F. Still<sup>(6)</sup> in his recent Lumsden lectures without considering the possibility that it also may owe its origin to similar dietetic deficiencies. Its absence in breast-fed children, its onset between the age of nine months and two years, the diarrhoea which so frequently precedes it, the cessation of growth, the ill-formed pale 'catmeal' stools, the frequent association of scorbutic symptoms, the abdominal distension, the afebrile nature of the malady, the diminished size of the liver, the blood changes, the occurrence of cedema, the thin bones, the muscular feebleness—all these find their counterpart in pigeons fed on an exclusive dietary of autoclaved milled rice<sup>(4)</sup>. The pathological findings recorded in this paper indicate with what readiness pathogenic organisms may invade the atrophying intestinal mucous membrane and lead to inflammatory states and fibrotic thickenings therein. The enlargement of the adrenals, which is so constant a result of 'vitaminic' starvation, and so frequently associated with cedema in birds, may not be wanting in coeliac disease; I can find, in the literature available to me, no records with regard to the adrenals in this malady.

I venture to suggest that those who have the opportunity should examine their cases of coeliac disease, and more especially all available *post-mortem* material, from the point of view of food deficiency. Whether or not this malady is found to be due primarily to such deficiency, it would appear probable that a number of its characteristics are secondary to the imperfect assimilation of these essential ingredients of the food.

If, now, we consider the possibility of sub-minimal 'vitaminic' supply protracted over prolonged periods from childhood onwards, we have, I think, a new view-point from which to consider the genesis of that common disorder :

### III. CHRONIC INTESTINAL STASIS.

In the recent edition of Sir Arbuthnot Lane's work on this subject<sup>(7)</sup>, Professor Arthur Keith draws attention to two anatomical factors which he regards as of primary importance in the causation of chronic intestinal stasis : (1) Defective action on the part of the abdominal musculature, and (2) a lesion of the neuro-muscular system of the intestine.

The histo-pathological findings recorded in this paper indicate one means by which both the abdominal musculature and the neuro-muscular system of the intestine may be simultaneously impaired in functional capacity. Deficiency of certain accessory food factors leads to atrophy of all muscular tissue as well as to disordered function or actual degeneration of motor nerve cells throughout the entire body. The abdominal musculature and the nerve elements controlling it must of necessity suffer along with other muscular and nervous tissues. It may be concluded, then, that defective action on the part of the abdominal musculature will ultimately result in consequence of deficiency of accessory food factors.

In addition to this functional defect on the part of the abdominal wall, we find also in pigeons and in guinea-pigs starved of 'vitamines' unquestionable evidence in the wall of the intestine itself of neuro-muscular lesions of great significance. Whether or not we are justified in applying these findings to the genesis of stasis in the human subject, we may at least regard with suspicion human food which does not conform to standard in respect to perfect 'vitaminic' balance.

There can be no doubt that the dietaries of children amongst the poorer, and often amongst the richer, classes are frequently deficient in these important ingredients. Subsistence on such ill-balanced dietaries from childhood onwards may be regarded as calculated to lead to defect of the abdominal musculature, to neuro-muscular lesions of the intestine and to changes in the glandular elements of its mucous membrane. Indeed, the pathological changes in the large bowel in stasis, as described

by Professor Keith, are strikingly similar to those I have enumerated as occurring in pigeons fed on a 'vitaminic'-free food. He describes the morbid changes as affecting two systems—the glandular and the neuromuscular: 'The chief change in the muscular coats of the diseased great bowels removed from cases of enterostasis is a fibrosis—one which mainly affects the tissue between the outer and inner coats of the bowel, the intermediate stratum which insheathes the myenteric plexus. Degeneration in areas of the musculature, particularly of the outer longitudinal coat, also occurs. In every case there are inflammatory changes varying in degree. There are many marked changes in the lining glandular epithelium and in the submucous coat. One notes also that there is engorgement of the subperitoneal vessels, and that the subperitoneal tissue is much thicker and denser than is normal.' Having regard to the fact that the pathological processes I have described are acute in nature, and induced by intensive 'vitaminic' deprivation, whereas those recorded by Professor Keith are chronic, the similarity between them suggests that sub-minimal 'vitaminic' supply protracted from an early age has a fundamental influence in causing the lesions found in stasis. However this may be, we must regard with suspicion an influence which is capable of providing at once the anatomical conditions necessary for the development of stasis and the facilities for systemic infections which are known to result from it.

#### OBSERVATIONS ON THE EFFECTS OF 'VITAMINIC' SUBSTANCES AS CURATIVE AGENTS IN 'POLYNEURITIS GALLINARUM.'

In the course of the present research, many observations have been made with regard to the curative action of 'vitaminic' extracts, and also of such whole grains as *mung dal* in the morbid state induced in pigeons by an exclusive dietary of autoclaved rice. Since these therapeutic trials bring into prominence certain points of interest, the following illustration may be given:

Two young pigeons developed *polyneuritis gallinarum* in typical form and in severe degree as a result of an exclusive diet of autoclaved rice and butter. Both birds presented pronounced cerebellar symptoms and astasia; both had severe convulsive seizures turning 'cart-wheels' backwards at frequent intervals(?). At this stage, the birds were artificially fed with the *dry whole grains of mung dal* (a species of pea). Within twenty-four hours the convulsions had ceased, the cerebellar



symptoms had disappeared, and both birds were able to walk, although with a high-stepping and uncertain gait. They improved hourly on this dietary, both eating greedily of it. After the lapse of seventy-two hours one died suddenly. An autopsy was performed immediately; the whole gastro-intestinal tract was greatly engorged, the bowel walls were very thin and the upper four or five inches of the bowel distended with partly digested grain, the lower bowel being practically empty. It was noted that the contents of the upper bowel were not of that creamy consistency which is usual in health, but resembled rather a coarsely-ground collection of the ingested grain. The transport of the intestinal contents along the bowel lumen was delayed. Histological examination revealed pronounced changes in the bowel walls. The heart's blood yielded no growth by aërobic methods of culture.

The other bird survived. It was placed subsequently on mixed grains, and with the exception of a high-stepping and somewhat unsteady gait it exhibited after four days no symptoms referable to the nervous system. It appeared to be perfectly restored to health within ten days.

In connexion with this illustration I would direct attention to the following facts:

(1) The nervous symptoms present in *polyneuritis gallinarum* may be rapidly ameliorated, or even recovered from, and yet the bird die in consequence of gastro-intestinal lesions or (as I have found in other cases) of hæmic infections. This observation has, I think, a significant bearing on work designed to determine the 'vitaminic' value of food-stuffs by means of curative experiments in pigeons suffering from *polyneuritis gallinarum*.

(2) The rapid recovery from nervous symptoms indicates that the nervous system is, of all others, the least affected as regards organic lesions. In this system the symptoms are mainly the result of functional disorder. In the intestine, on the other hand, a greater destruction of tissue cells takes place. The intestinal lesions will consequently be recovered from more slowly.

(3) The remarkably rapid disappearance of convulsive seizures, of astasia and of cerebellar symptoms indicates that accessory food factors, if they do not actually activate or energize the nerve cells, at least they complete the circuit of nervous current. In their absence the large mass of nerve cells is incapable of normal activity; their provision establishes relatively normal cellular activity with dramatic suddenness.



Their action has been compared by Bayliss to that of catalytic substances; my own experimental experiences lead me to a like view.

(4) Solutions of 'vitaminic' substances (such for example as a solution in water of the dried alcoholic extract of the yolks of eggs) will, when administered to birds suffering from avian beri-beri, often cause recovery to take place with extraordinary rapidity. In cases, such as are recorded in the above illustration, recovery from nervous symptoms was quickly brought about by artificial feeding with the small Indian pea known as *mung dal*. The question then arises as to whether the liberation of accessory food factors from the dry hard seeds of *mung dal*, in quantity sufficient to bring about so rapid an amelioration of nervous symptoms, is wholly due to the ordered processes of digestion. It is hardly credible that a gastro-intestinal tract, in which are present the grave pathological changes I have described, could complete the processes of digestion with sufficient despatch to secure so rapidly beneficial a result. Indeed, in the bird which died digestion was far from complete, the bowel contents were abnormally delayed in transit and histological examination revealed an extreme degree of pathological change in the bowel walls. One is led to surmise, therefore, that some process more rapid than that of digestion may be concerned in the liberation of these substances—a process comparable, it may be, to the generation of growth ferments in seeds planted in warm moist earth.

#### CONCLUSIONS.

1. Dietaries deficient in accessory food factors give rise in pigeons and in guinea-pigs to congestive and atrophic changes in all coats of the bowel, to lesions of its neuro-muscular mechanism, to impairment of its digestive and assimilative functions, and to failure of its protective resources against infection.

2. The functional perfection of the gastro-intestinal tract is dependent in considerable measure on the adequate provision of accessory food factors derived from fresh fruit and vegetables.

3. Certain gastro-intestinal disorders in the human subject—of which three examples are referred to—may owe their origin to the long-continued sub-minimal supply of accessory food factors.

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# THE PATHOGENESIS OF DEFICIENCY DISEASE.

## NO. IV. THE INFLUENCE OF A SCORBUTIC DIET ON THE ADRENAL GLANDS.

BY

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THE influence of a scorbutic diet of crushed oats and autoclaved milk on the adrenal glands was studied in guinea-pigs.

The animals were isolated in separate cages. The experiment was continued until death occurred. Autopsies were performed with the attention to detail previously described.<sup>(1)</sup> Aërobic cultures of the heart's blood at autopsy gave negative results in four cases, positive results in one (No. 5, Table 1). In this case the organism isolated failed to kill guinea-pigs on subcutaneous injection in large dosage.

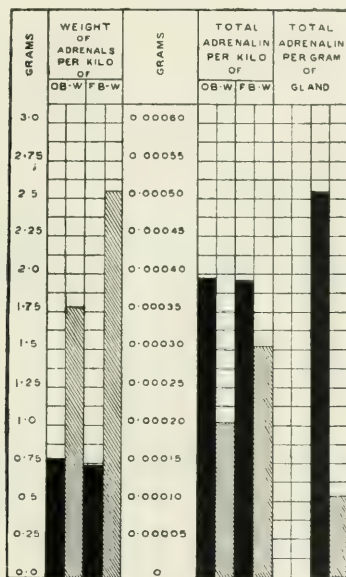
### MORBID ANATOMY.

Increase in size of the adrenal glands together with greater or lesser degrees of congestion were the main naked-eye changes observed. The yellow colour of the organs was usually found to have given place to a reddish-yellow tinge. The superficial vessels presented varying degrees of engorgement. Minute hæmorrhagic effusions under the serous coat were frequently encountered.

The weight of the adrenals of guinea-pigs dying in consequence of the scorbutic diet was approximately double that of health (Table I). Whereas the gross weight of both organs in healthy guinea-pigs ranged between 0.390 and 0.520, with an average weight of 0.467 grams, in

guinea-pigs fed on the scorbutic dietary the weight of both glands ranged between 0.850 and 1.150 grams, with an average weight of 0.955 grams. The increase in weight due to the scorbutic diet was even more marked when the weight of the glands was calculated per kilo of *original* and of *final* body-weight (Chart I).

CHART I.



Char I. Showing weight of adrenals and total adrenalin per kilo of original (OB-W) and of final (FB-W) body-weight, also total adrenalin per gram of gland in (a) healthy guinea-pigs (black columns) and in (b) guinea-pigs fed on a scorbutic diet (shaded columns).

#### ADRENALIN-CONTENT OF THE ENLARGED GLANDS.

The adrenalin-content was estimated by the method of Folin, Cannon and Dennis.<sup>(3)</sup> In each case one gland was used for adrenalin-

estimation, the other for histological study. On the presumption that both organs were affected in like degree, the total adrenalin-content of both adrenals was calculated from the results obtained with one gland. In some instances the right gland was used for adrenalin-estimation, in others the left. The results of these estimations are shown in the following table (Table I), and are illustrated graphically in Chart I.

TABLE I.

*Showing the adrenalin-content of the adrenal glands, the total adrenalin per kilo of body-weight, etc., in (a) five guinea-pigs fed on a scorbutic diet, and (b) in four healthy guinea-pigs fed on normal food.*

Number of guinea-pig.	Original weight of guinea-pig in grams.	Final weight of guinea-pig in grams.	Weight of adrenals in grams.	Weight of adrenals per kilo of original body-weight in grams.	Weight of adrenals per kilo of final body-weight in grams.	Total adrenalin in both adrenal glands in grams.	Total adrenalin per kilo of original body-weight in grams.	Total adrenalin per kilo of final body-weight in grams.	Total adrenalin per gram of gland.	Experimental or control animal.
1	2	3	4	5	6	7	8	9	10	11
1	550	390	.850	1.545	2.179	.000113	.000205	.000289	.000132	Experimental.
2	520	400	1.029	1.978	2.572	.000143	.000275	.000357	.000139	Do.
3	500	320	.850	1.700	2.656	.000096	.000192	.000300	.000112	Do.
4	550	320	.900	1.636	2.812	.000116	.000210	.000362	.000128	Do.
5	520	390	1.150	2.211	2.948	.000086	.000165	.000220	.000074	Do.
	528	364	.955	1.814	2.633	.000110	.000209	.000305	.000117	Averages.
7	570	620	.390	.684	.629	.000253	.000443	.000408	.000648	Control.
8	610	610	.520	.852	.852	.000216	.000354	.000354	.000415	Do.
9	610	610	.460	.754	.754	.000253	.000414	.000414	.000550	Do.
10	670	670	.500	.746	.746	.000233	.000347	.000347	.000466	Do.
	615	627	.467	.759	.745	.000238	.000389	.000380	.000519	Averages.

NOTE: I am indebted to Colonel Cornwall, I.M.S., for the figures relating to guinea-pigs Nos. 8, 9 and 10. Owing to difficulty in obtaining supplies of pure anhydrous phosphoric acid and of sodium-tungstate I was unable to estimate the adrenalin-content of the adrenal glands in my controls to this experiment (with the exception of Pig No. 7). The figures given for cases Nos. 8, 9 and 10 are the results of estimations made by Colonel Cornwall for another purpose in three healthy guinea-pigs from laboratory stock.

## ANALYSIS OF TABLE I.

(1) The total quantity of adrenalin in both glands in guinea-pigs fed on a scorbutic diet was less than half that present in healthy guinea-pigs (Table I, col. 7) although the weight of the organs was more than twice as great in the former as in the latter. (Table I, cols. 4, 5, 6.)

(2) Similarly the total adrenalin per kilo of *original* body-weight (*i.e.*, the weight of the animal before the experiment commenced) was little more than half that of health. (Table I, col. 8.)

(3) When calculated against the *final* body-weight (Table I, col. 9) of the guinea-pig, the total adrenalin per kilo was found to be less markedly reduced; that is to say, the total adrenalin-production does not diminish in proportion to the diminution of body-weight.

(4) The total adrenalin per gram of gland was less than one-fourth of that found in health. (Table I, col. 10.)

(5) It will be noted that in guinea-pig No. 5, in which a coliform organism was cultured from the heart's blood at autopsy, the adrenalin-content was considerably less than in the other four animals in which no infection was detected by *aërobic* methods of culture.

A pronounced reduction in the amount of adrenalin in the suprarenal glands was thus found to result in guinea-pigs from a scorbutic dietary of crushed oats and autoclaved milk.

In connexion with these findings it is of interest to direct attention to the fact that the total adrenalin per gram of gland in healthy pigeons is ten times greater (·0023 grams) than in healthy guinea-pigs (·00023 grams). This greater proportion of adrenalin in birds may bear some relationship to the fact that they excrete uric acid as such. The work of Bauer,<sup>(5)</sup> which leads him to believe that 'the function of the adrenal cortex is the making of pigment from the superfluous uric acid in the blood,' is of interest in this connexion. He considers that the pigment, which he believes to be made by the cortex, is transformed by the medulla into adrenalin.

A further point of interest in connexion with these findings is the contrast they afford to the great increase in the adrenalin-content of the adrenal glands which occurs in pigeons wholly deprived of accessory food factors of all classes. In another place<sup>(4)</sup>, I have shown that the adrenalin-content of these organs in birds is largely dependent on the class of accessory food factor which is absent from the dietary.

## HISTO-PATHOLOGY.

The histological changes in the adrenal glands which result from a scorbutic dietary comprise :—

- (1) Hæmorrhagic infiltration, and
- (2) Degenerative changes in the cellular elements of the medulla and cortex.

*Hæmorrhagic infiltration.*—There are two features of importance in connexion with the hæmorrhagic infiltrations :—

- (1) Their circumscribed character and situation in the adrenal cortex ; and
- (2) the fact that they may occur before any *clinical* evidences of scurvy are observed, and even before any notable scorbutic changes are to be found at autopsy between cartilage and rib.

The circumscribed character of the hæmorrhagic infiltrations and their situation in the adrenal cortex are shown in Fig. 1. A higher magnification of a similar section is shown in Fig. 3. Through the kindness of Colonel Cornwall, I.M.S., I am enabled to show photomicrographs of sections of the adrenal from a case of street-virus rabies in a guinea-pig. These (Figs. 8 and 9) illustrate the radiating distribution of the capillary spaces of the adrenal cortex in guinea-pigs. They provide also an instructive contrast to the sections of adrenals from guinea-pigs fed on a scorbutic diet. It will be noted that in the latter the congestive process is not confined to engorgement of the capillary spaces of the cortex but results in more or less circumscribed hæmorrhagic infiltration of scattered areas of the cortex. These hæmorrhagic areas are of varying sizes ; their more or less uniform distribution around the periphery of the cortex is their characteristic feature. I have not observed this curious distribution of hæmorrhages in the adrenal glands of guinea-pigs dying of infectious diseases in which both cortex and medulla are often extensively congested. The hæmorrhagic areas were distributed more or less uniformly around the periphery of the cortex in three cases in the present series (Figs. 1 and 3). They were present on only one side of the adrenal cortex in one case. In the fifth case (Fig. 5) they were absent, a moderate degree of generalized hæmorrhagic infiltration of the cortex alone being present.

The changes in the cortical cells consist in (a) loss of their tessellated appearance, (b) vacuolation and disintegration of cells, and



PLATE XI.



Fig. 1.



PLATE XII.



Fig. 2.

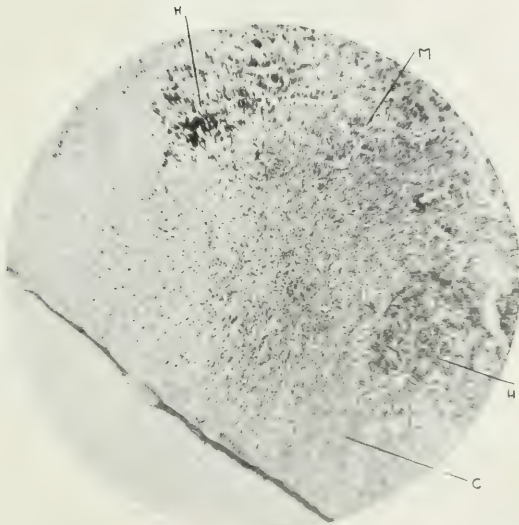


Fig. 3.



PLATE XIII.

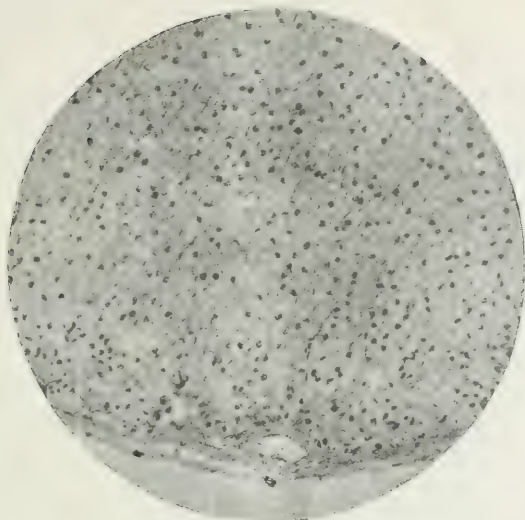


Fig. 4.

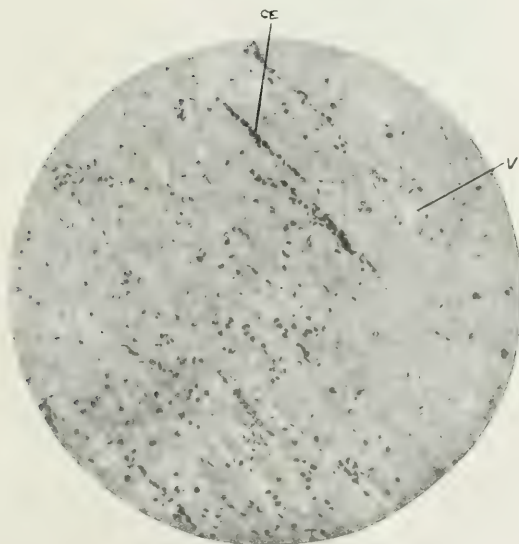


Fig. 5.



PLATE XIV.

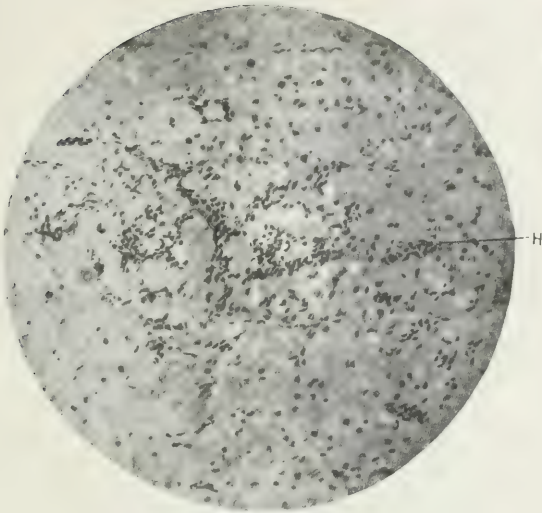


Fig. 6.

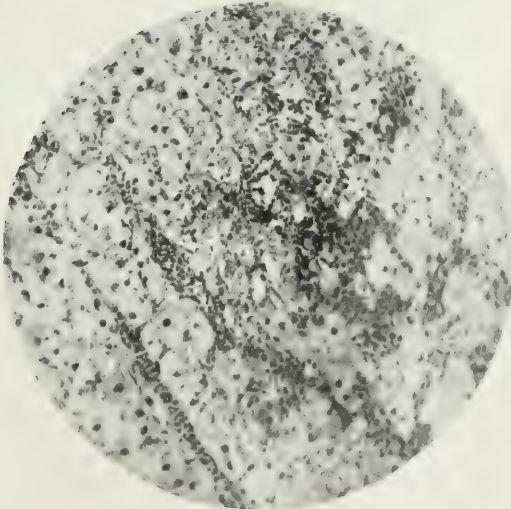


Fig. 7.



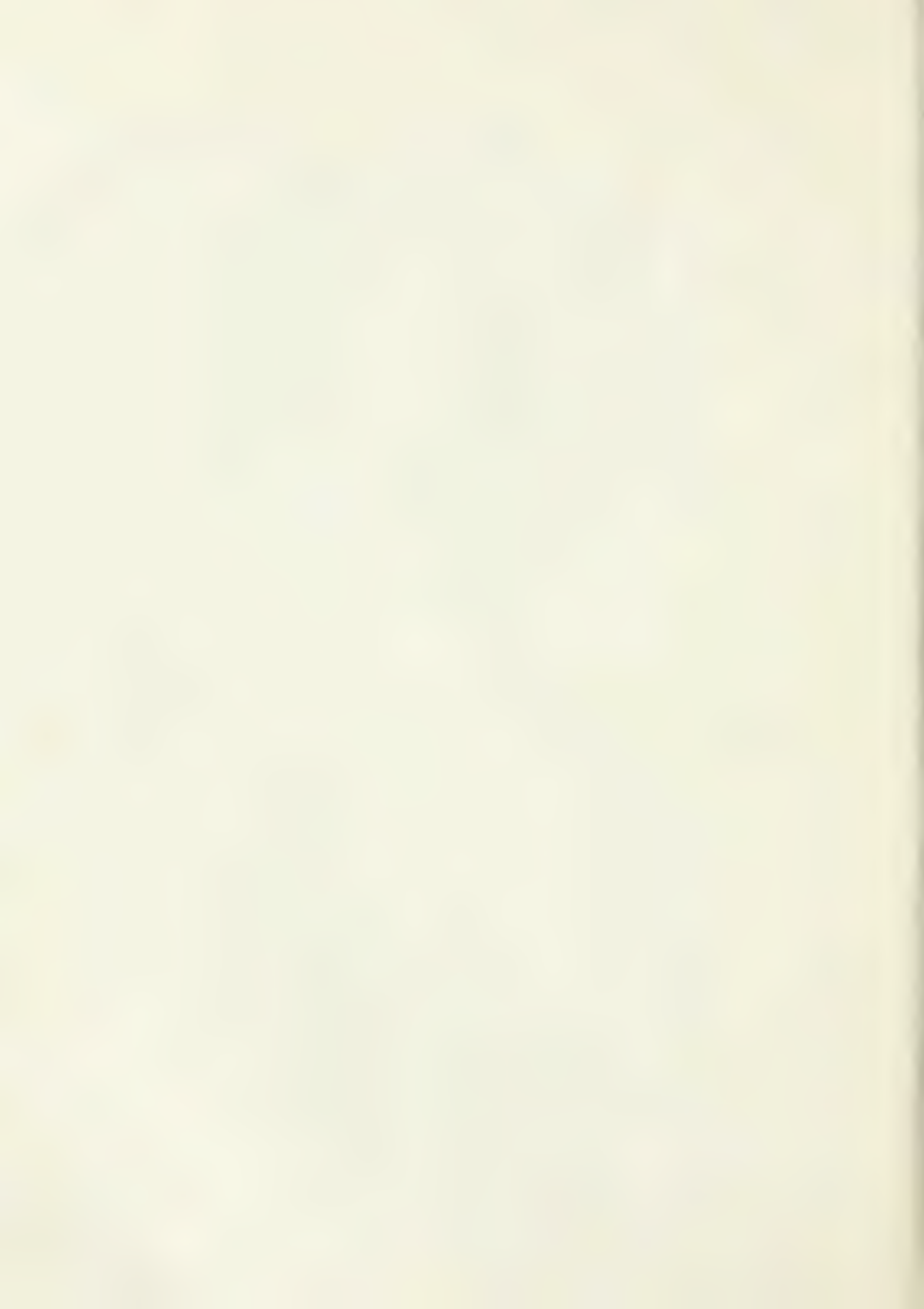


PLATE XV.

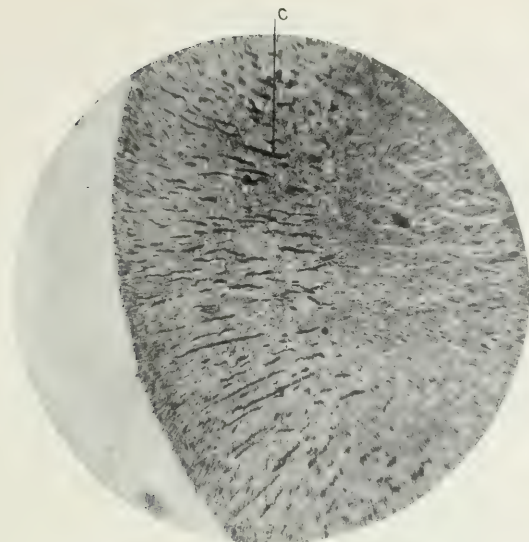


Fig. 8.

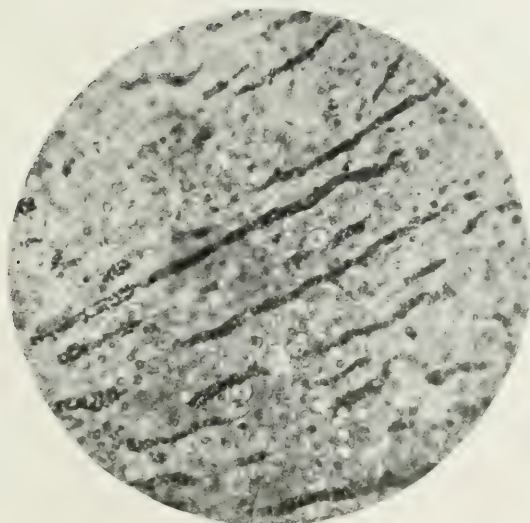


Fig. 9.



(c) disappearance or loss of staining reactions of their nuclei. These changes are well seen in the photomicrographs which illustrate three different cases; they call for no further description.

The medulla is much disorganized and shows cellular disintegration with scattered blood cells lying throughout its substance. Usually it is possible to detect in healthy glands the chrome-staining granules of the medullary cells. In the case of guinea-pigs fed on the scorbutic dietary these granules are few or altogether wanting.

I have been much impressed with the fact that the histo-pathological changes I have described were present in the adrenals of guinea-pigs, fed on the scorbutic dietary, which exhibited during life no clinical evidences of scurvy. Depression of adrenal function may, therefore, be regarded as a pre-scorbutic manifestation of a dietary deficient in accessory food factors of the 'C' class.

#### SUMMARY.

A scorbutic diet gives rise in guinea-pigs to—

- (1) an increase in size and in weight of the adrenal glands;
- (2) a marked diminution in the adrenalin-content of these organs;
- (3) hæmorrhagic infiltration of the adrenal glands, usually circumscribed in extent and situated around the periphery of the adrenal cortex; and to
- (4) degenerative changes in the cellular elements of the adrenal cortex and medulla.

#### CONCLUSIONS.

1. A scorbutic diet causes pronounced depreciation in functional capacity of the adrenal glands in guinea-pigs.

2. The impairment of adrenal function occurs before clinical evidences of scurvy manifest themselves.

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# NOTES ON A MONKEY PLASMODIUM AND ON SOME EXPERIMENTS IN MALARIA.

BY

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SCHAUDINN'S hypothesis that relapse in malaria is due to a reversion of the female gametocyte to the malarial ring by a process of parthenogenesis is well known. The most recent and most valuable contribution to this problem is probably that of J. D. Thomson (1917), who claims that Schaudinn's figures illustrating parthenogenesis of the B. T. macrogametocyte in reality only illustrate combinations of gametocyte plus schizont within the same erythrocyte.

It has always seemed to the writer that the best method of testing the truth or otherwise of Schaudinn's hypothesis would be by either (a) cultural or (b) animal experiment. The success of Mesnil and Roubaud in June 1917 in transmitting benign tertian malaria to a chimpanzee, seemed further to bring such animal experiments within the bounds of possibility. The following summary of some negative experiments in this connection may therefore be of interest, as they led up to the discovery of the monkey plasmodium to be described.

## *Experiment I.*

On 18th November, 1917, a patient presented himself at the Institute with a history of chronic malaria of some years' standing and an enlarged spleen. Films showed the presence of numerous M. T. rings and crescents in the peripheral blood.

3 c.c. of the blood was taken from the median basilic vein at 3 P.M., and distributed into four tubes of N. N. N. media. These were incubated (a) two at 40°C; and (b) two at 22°C. The contents of the tubes were examined daily for the next 14 days. The only change seen was gradual disintegration of the parasites.

On the same day at 4 P.M. the patient had a rigor, 10 c.c. of blood was immediately aspirated into 10 c.c. of citrated saline in a 20 c.c. Roux syringe, and 10 c.c. of the mixture given at once subcutaneously in the abdominal wall to each of two small *Macacus* rhesus monkeys. Both monkeys however remained perfectly fit and daily examinations of the blood for the next 23 days gave negative results.

### *Experiment II.*

On 25th November, 1917, another patient presented himself for treatment for chronic malaria and was placed on daily intravenous injections of quinine together with quinine orally. This patient was under almost daily observation until the 13th December, 1917; *i.e.*, for 19 days. During this period in spite of full quinine treatment his blood films showed a persistent crescent infection. No schizonts or trophozoites were seen at any time. The results of a complete examination of a thin blood film upon each occasion were as follows:—

Date.	Total number of crescents.	Macrogametocytes.	Microgametocytes.
25-11-17 ..	27	23	4
27-11-17 ..	56	49	7
28-11-17 ..	29	25	4
29-11-17 ..	19	18	1
30-11-17 ..	45	41	4
1-12-17 ..	31	29	2
2-12-17 ..	26	25	1
3-12-17 ..	3	3	0
7-12-17 ..	16	15	1
11-12-17 ..	14	14	0
12-12-17 ..	4	4	0
13-12-17 ..	9	8	1



191



1



2



3



4



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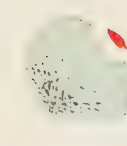
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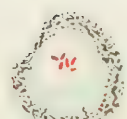
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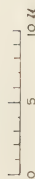
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The above figures are of interest as showing (a) the failure of quinine, even when given in full doses intravenously plus orally, to exterminate gametocytes; and (b) the preponderance of female over male gametocytes (254 to 25 in 12 counts) in a residual infection.

On the afternoon of the patient's arrival it so happened that there was available at the Institute a hanuman monkey—(*Sp. Semnopithecus entellus*). This animal had been at the Institute since the preceding January, had never been used experimentally, and had always been in excellent health.

2 c.c. of blood from the patient's median basilic vein were aspirated into 10 c.c. of citrated saline at 3.30 P.M., on the 25th November, 1917. Under ether anaesthesia the right femoral vein of the monkey was exposed by operation and the contents of the syringe slowly injected into the vein.

At 2.30 P.M. next day—23 hours later—the monkey was clearly ill. The rectal temperature was only 98.5°F. instead of a normal temperature of 101.8°F. Coma and prostration developed; the rectal temperature sank to 95.6°F. at 6 P.M.; to 95°F. at 9 A.M. on the 27th; and the monkey died 48 hours after the injection, at 3.30 P.M. on the 27th.

Figures 1 to 16 in the attached plates are from the monkey's peripheral blood 23 hours after injection; figures 17 to 29 are from the same at 27 hours; figures 30 to 39 at 40 hours; and figures 40 to 50 at 44 hours,—just before death.

A *post-mortem* examination was made immediately after death. Cultures on agar from the cardiac blood remained sterile. The internal organs were apparently normal, except the spleen which was very acutely congested, and the bone marrow (of the femur) which was also congested. The site of operation showed no unusual feature and no hæmorrhage. Figures 51 to 79 are from the cardiac blood and figures 80 to 93 from spleen smears. Figures 1 to 50 (peripheral blood) are all from air-dried slides; figures 51 to 93 from slides fixed whilst still wet with Schaudinn's fixative. The stain throughout was Leishman's stain; as the only Giemsa stain available at the Institute has proved unsatisfactory.

The state of affairs seen in the films was most extraordinary. In the cardiac blood films the majority of erythrocytes were infected with a plasmodial organism, and some cells showed double infection. At first sight this infection appeared to be very similar to that of *M. T. malaria* in man: figures 15, 25, 26, 46, 55, 77. It was, however, inconceivable that a crescent could have reverted to a ring in 23 hours (figure 15).

All available literature was immediately searched for descriptions of the natural plasmodia of monkeys. It is this search which is the writer's reason for putting forward this paper, as he has been unable to obtain anywhere any good drawings or descriptions of such parasites. Further, no mention has apparently been made of any plasmodium in the hanuman.

It is clear that the parasite had no connection with human malaria or with the M. T. crescents injected into the monkey; but that it is a plasmodium peculiar to the monkey species concerned, and the name *Plasmodium semnopithecii* is suggested.

*P. semnopithecii* of the hanuman monkey shows resemblances to both the B. T. and M. T. parasites of man. In its early form it is an almost non-pigmented ring, closely resembling that of M. T.: figures 15, 25, 26, 46, 55, 77. Some of the rings, however, are larger and more flimsy and more resemble the ring of B. T.: figures 24, 30, 42, 56, 58, 76. The gametocytes rather recall those of the B. T. parasite: figures 2, 5, 8, 33, 47.

The peculiar feature of the parasite, however, appears to be the existence of innumerable free forms: some of them undergoing schizogony whilst still extra-cellular: figures 11, 12. The free forms appear to have a definite cycle of development, as follows:—

- (1) in the chromatin becoming marginal and linear: figures 3, 4, 6, 13, 64;
- (2) in the formation of a 'brilliant vacuole' between the marginal chromatin and the main body of the parasite: figures 14, 17, 19, 21, 23, 27, 39, 43, 73;
- (3) after which the parasite appears to impinge against a red cell, with the chromatin always in first contact: figures 52, 69, 36, 31, 50, 61;
- (4) and then enters the red cell to become a typical ring; whilst the main portions of the cytoplasm and pigment are discarded and disintegrate: figures 31, 36, 59, 69, 72, 75.

At the same time, owing probably to the very heavy infection present, many parasites seemed in process of chromolysis, plasmolysis and destruction: figures 9, 28, 35, 49, 66, 67. The forms seen in the spleen smears—figures 80 to 93—all seem to be undergoing disintegration and even non-nucleated masses of parasitic cytoplasm are



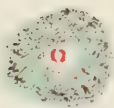
PLATE XVIII.







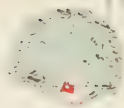
80



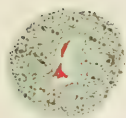
81



82



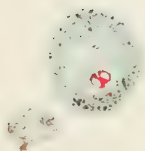
83



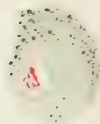
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85



86



87



88



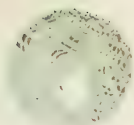
89



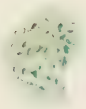
90



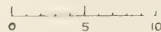
91



92



93





present: figures 86, 93. The spleen appears to be the organ of destruction of the parasite rather than its reservoir.

Experiment II may be summarised by saying that the shock of anæsthetic, operation and the injection of foreign blood had stimulated a latent infection of a plasmodium peculiar to the hanuman species concerned, and that the infection had suddenly become virulent and fatal. If the 'free forms' illustrate parthenogenesis at all, which is very doubtful, it is parthenogenesis of this plasmodium only. Three figures—Nos. 1, 7, 8—suggest commencing nuclear division in a macrogametocyte.

The writer would be only too glad to secure further specimens of the hanuman monkey, but more than a year of correspondence has hitherto resulted unsuccessfully.

### *Experiment III.*

Immediately after the death of the monkey in Experiment II peripheral blood films were searched from all the other monkeys at the Institute—12 in number and all of species *M. rhesus*. No parasites were found. Two monkeys, who were apparently in excellent health, were selected and films of the peripheral blood examined daily from each for 16 days without finding any parasite.

By 11th December, 1917, it was certain (a) that the patient of Experiment II was a purely gametocyte carrier free from schizonts and trophozoites in the peripheral blood; and (b) that both monkeys contained no natural infection. On this day the patient's blood showed 440 crescents per c.mm., as counted in a Thoma Zeiss hæmatocytometer cell. 10 c.c. of the patient's blood was aspirated into 10 c.c. of citrated saline in a 20 c.c. Roux syringe. The right femoral vein of each monkey was exposed by operation under ether anæsthesia. It was found that the only satisfactory method of injecting even so large a vein in so small a monkey was by fitting a broken 1 c.c. needle to the syringe. The following intravenous injections were given:—

To Monkey No. 1, 5 c.c. of mixture, containing  $2\frac{1}{2}$  c.c. of blood; or 1,100,000 crescents.

To Monkey No. 2, 15 c.c. of mixture, containing  $7\frac{1}{2}$  c.c. of blood; or 3,300,000 crescents.

The blood mixture was in the syringe for 15 minutes before the injections were completed. Immediately afterwards the last drop

in the syringe was examined microscopically. A few crescents only showed enlargement and partial rounding up of the centre, and one showed pigment collected at only one pole; but the majority were perfectly normal. The R. B. Cs. were not at all hæmolyed.

Blood films from each monkey were examined for the next 14 days; but no malarial or other parasites were met with at any time. The monkeys remained in excellent health and showed normal temperatures— $101.8^{\circ}$  to  $102.6^{\circ}\text{F}$ . At 24 hours the films from Monkey No. 2 showed a great deal of granular platelet debris and also clear clusters of free malaria pigment, but no other change.

On the conclusion of Experiment III it became clear that if further experiments were to be conducted it was essential (a) to use anthropoid apes as being possibly more susceptible to human malaria; and (b) to devise some method of collecting crescents in enormous numbers suitable for the intravenous injection of massive doses in small volumes of fluid.

In the summer of 1918, through the kindness of W. H. Woodward, Esq., Hoogrijan, an adult specimen of the common anthropoid of Assam,—*Hylobates hoolock*—was secured and placed under observation with a view to the repetition of the experiment. Unfortunately the carelessness of an animal attendant allowed this most valuable animal to escape, and it has hitherto proved impossible to procure another. One or two adults of this species would be most gratefully received.

During 1918 several series of experiments were carried out towards the elucidation of problem (b). As M. T. crescent carriers are few and far between, work was commenced on the halteridium parasite of the pigeon by way of preliminary. These experiments are not worth detailed record; but as it is obvious that any method of greatly concentrating blood parasites would much facilitate diagnosis in such conditions as kala-azar, latent malaria, etc., where parasites in the peripheral blood are few and far between, a brief résumé of results obtained may perhaps be given. Citrated or defibrinated parasite-containing blood was used. The methods employed were: (a) to try and secure complete hæmolysis of all erythrocytes, so as to leave only residues of stromata and liberate the parasites; (b) to centrifuge heavily so as to collect together into a small volume all parasites present; (c) to wash the deposit and centrifuge repeatedly with normal saline; and (d) to examine fresh and stained thick films of the centrifuged deposit. This part of the work was heavily

handicapped by the want of electric power and of an electric centrifuge at the Institute.

The hæmolytic reagents employed were as follows, together with notes on some results obtained :—

*Class 1. Physical reagents.*

Alternate freezing and thawing.

Distilled water.

Distilled water, immediately followed by salt to make normal saline.

*Class 2. Chemical reagents.*

$\frac{1}{2}$  per cent HCl in distilled water and in saline.

$\frac{1}{2}$  per cent acetic acid in distilled water and in saline. The latter yielded good results as far as collecting parasites was concerned; but the parasites stained badly and were clearly not living.

Saturated and half-saturated solution of ether in distilled water and in saline.

Saturated and half-saturated solution of chloroform in distilled water and in saline. Saturated chloroform in saline yielded fair results.

10 per cent alcohol in saline.

0·3 per cent sod. taurocholate in saline and in distilled water.

0·2 per cent ammon. oxalate in distilled water; yielded good results but the parasites were not living.

Ox bile.

Ox bile with ether and with chloroform to saturation.

*Class 3. Sera (used always with fresh guinea-pig complement).*

Fresh sheep serum.

Fresh goat serum.

Fresh ox serum.

Anti-pigeon immunised rabbit sera. Results excellent. In one experiment with defibrinated blood the pigeon's blood before experiment showed 4,000 crescents per c.mm.; whereas the deposit after experiment gave a content of 64,000 crescents per c.mm. The parasites stained well.

Ditto; with ether and with chloroform to saturation and to half saturation. Results bad.

Anti-pigeon immunised goat sera. When pooled yielded a minimal hæmolytic titre of only 1 in 20.

Anti-human immunised goat sera. Yielded sera of only inferior hæmolytic titre.

Anti-human immunised gander sera. When pooled yielded a minimal hæmolytic titre of 1 in 300; and may be of value in future work.

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# THE ASSOCIATION OF THE BACILLUS OF HOFFMANN WITH DIPHTHERIA IN INDIA.

BY

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IN a previous paper (1918) the writer has given an account of an outbreak of diphtheria at a boys' school in Shillong in 1917. In 1918 the disease recurred in the station and was focussed in and around a girls' school. Concurrently with the 1918 outbreak in Shillong was a limited outbreak at Gauhati, which preceded the Shillong outbreak, and from which the infection was almost certainly re-imported into Shillong. At the moment of writing (8-3-19), the disease has again recurred simultaneously in both stations, and the outlook for the season of 1919 is not promising. The figures for both stations for the two years, 1917-18, are given in Table I, and give a total case and carrier incidence in accordance with the findings of other workers in other epidemics. By the term 'case' is meant a patient showing the clinical picture of diphtheria; by the term 'carrier' a person in whose throat the true Klebs-Löffler bacillus was discovered, but who showed no clinical signs of disease.

## GENERAL NOTES ON THE TWO OUTBREAKS.

A few notes on the general features of the two outbreaks may be worth placing on record:—

### 1. *Meteorology.*

Both outbreaks were associated with the period of the rains whereas during the intervening cold and hot season, 1917-18, both stations were almost free from the disease.

2. *Race and sex and age incidence.*

The case distribution was as follows :—

Europeans: boys 10; girls 10; adult males 1; adult females 4.

Total 25.

Indians: boys 6; girls 0; adult males 4; adult females 1.

Total 11.

The carrier distribution was as follows :—

Europeans: boys 16; girls 13; adult males 2; adult females 2.

Total 33.

Indians: boys 1; girls 1; adult males 0; adult females 1.

Total 3.

3. *Foci of infection.*

The apparent foci of infection were as follows (Tables II and III) :—

- (a) The 1917 outbreak was perhaps imported from Calcutta. Cases 1 and 2, brother and sister, came from Calcutta; but had been in Shillong for two months before developing the disease. The boy, Case 1, had been attending the boys' school.
- (b) Carrier 24, February 1918, had been nursing a case of diphtheria in Calcutta, and the throat was found positive on her return to Shillong immediately afterwards. All precautions were taken, and this focus was apparently of no importance.
- (c) Case 15, May 1918, developed the disease shortly after arrival from Gauhati. The four immediate contacts were all negative. He continued to show Klebs-Löffler bacilli until his departure from Shillong for Gauhati in June 1918. On 19-2-19, when diphtheria recurred at Gauhati, his throat was again examined. He was in excellent health; but the culture showed very scanty diphtheria colonies, with a profusion of colonies of the bacillus of Hoffmann. This case was probably not concerned with the 1918 outbreak at Shillong, but may be of importance in connection with the 1919 outbreak at Gauhati.
- (d) The 1918 outbreak at the girls' school was apparently imported from Gauhati by a large family which came from there into Shillong in July 1918. An infant in this family had

died of diphtheria—(throat swab not examined)—and the family moved to Shillong. One of the daughters was teaching at the school. The father, one son, and this daughter were found to be carriers. (Carriers 26, 27 and 32.) Another daughter at the school developed the disease. (Case 22.)

- (e) Cases 25 and 26, mother and son, were in an Indian clerk's household in the immediate vicinity of the girls' school. The household had, however, no direct contact with the school.
- (f) The occurrence of sporadic cases and carriers, both among Europeans and Indians, in Shillong (Cases 9, 10, 11, 13, 14, 23, 24; Carriers 6, 12, 14, 16, 19, 21, 22, 23, 33, 34) shows that the infection has established itself in Shillong—as also probably in Gauhati—and that further sporadic outbreaks may be expected from time to time.

In brief, the history of the two years shows that the disease is endemic in both stations; and that the constant traffic between rail-head at Gauhati and motor terminus at Shillong and the movements of the population of summer visitors and school children, help to keep the infection current.

#### 4. *Contrast between 1917 and 1918.*

The 1917 outbreak in the boys' school was of insidious onset and was not at first recognised as diphtheria. Two deaths occurred out of 14 cases in 1917, and the carrier incidence of 13 per cent in the boys' school was fairly high.

The onset of the 1918 outbreak in the girls' school was met by the most prompt and vigorous measures by both the Civil Surgeon of Shillong and the Headmistress of the School. Examination of throats, segregation and disinfection were thoroughly carried out, and the following figures show an interesting contrast :—

Throat swabs sent for examination per individual—

From the girls' school, 1918 : 186 from 70 individuals; average 2·7 per person.

From the boys' school in 1917: 134 from 108 individuals; average 1·2 per person.

From outside households, 1917–18 : 192 from 163 individuals; average 1·2 per person.

In fact, whereas in 1917 the services of the laboratory were utilised chiefly for the identification of cases and carriers, in 1918 they were also utilised to ensure absence of infectivity before the discharge of cases and carriers. Finally after a last examination of all threats at the girls' school, the school was closed down and the pupils sent away at the end of October 1918.

In 1917 in the boys' school the carrier incidence was higher than the case incidence; for the girls' school in 1918 the reverse held. The 1917 outbreak was more virulent and more localised; the 1918 outbreak less virulent and more diffused. No deaths occurred in 1918; and it is to be noted that no cases occurred in the boys' school in 1918. The two outbreaks appear to have originated from different sources, and to have shown a different degree of virulence and infectivity.

#### 5. *Virulence of Klebs-Löffler strains examined.*

The virulence test was in all cases the injection of 2 c.c. of a 48-hour broth culture subcutaneously into an adult guinea-pig. Excluding bacilli of doubtful morphology and the bacillus of Hoffmann, 14 true Klebs-Löffler strains were thus tested; 8 from cases, 6 from carriers. Only one proved virulent (Carrier No. 11), and this came from a boy with chronically enlarged tonsils. The absence of virulence in the strains, however, is probably due to the fact that the test was chiefly applied to strains isolated from the throats of convalescent cases and persistent carriers (Cases 13, 15, 23; Carrier 24), as a rough test of the possible infectivity of the convalescent or carrier. The pressure of work at the height of both outbreaks prevented the testing of strains isolated at that time.

#### 6. *Cases clinically diphtheria, but bacteriologically negative.*

Cases 1, 2, 3, and 5 were only examined after convalescence, when they gave negative results. Case 11, twice negative, showed a typical membrane on the palate. Case 20 was clinically a very severe case of diphtheria at the girls' school: but cultures from 5 consecutive swabs gave negative results. Case 31, from Gauhati, was reported to be clinically diphtheria, whilst his brother, Case 32, showed typical Klebs-Löffler bacilli in a typically diphtheritic throat on the same day.

Thus in three cases clinically suffering from true diphtheria and examined at the time of most acute symptoms, the Klebs-Löffler

bacillus was not identified in culture on serum, despite careful examination of all possible colonies.

#### 7. *Technique of examination.*

The routine of examination in every case was the same. Very little importance was attached to the examination of the direct film, unless it resulted in a positive finding. In the later swabs Ponder's stain (Macalister, 1918) was used for the direct films with an improved percentage of positive findings. Even where the true Klebs-Löffler bacillus was seen in the direct film, however, cultures were always made for confirmation. Every swab was cultured immediately on receipt on inspissated serum, using Petri dishes for suspected cases, and slopes for the examination for carriers. The 18 to 24-hour culture was examined next day, colonies picked off, and stained by Neisser's, Cobbett's blue and Ponder's stains.

#### 8. *The Bacillus of Hoffmann.*

Tables II and III record the findings in all 208 swabs from the 36 cases and 36 carriers. When the typical beaded or polar Klebs-Löffler bacillus was present, the sign K.L. is given in the tables. Where an undoubtedly pure culture of the bacillus of Hoffmann was obtained, the sign H is used. Bacilli of doubtful morphology are shown by the sign  $\pm$ . The triple sign K.L.H. for a swab means that the culture yielded colonies of both the Klebs-Löffler bacillus and of the bacillus of Hoffmann.

Morphologically, at least, to make such a classification is easy. It is also of practical value if the  $\pm$  bacilli are returned as 'probable diphtheria'—whence the inclusion of Nos. 3 and 20 in Table III as carriers. The typical bacillus of Hoffmann is morphologically quite distinct from the typical Klebs-Löffler bacillus. The former is oval with pointed ends, and has a light mesial septum across its length; the latter is a rod, straight or slightly curved, and with rounded ends. The former has no granules, whereas the typical Klebs-Löffler bacillus shows deep blue-violet granules with Neisser's stain. The granules may be (a) only one at one pole; (b) one at each pole,—the commonest arrangement; or (c) there may be 3 or 4 throughout the length of the rod. In young serum cultures the true Klebs-Löffler bacillus never fails to show the well-known geometrical Chinese lettering or brushwood pattern; whereas the bacillus of Hoffmann in culture shows

bacilli arranged in parallel like the stakes in a fence. Films from a culture of the Klebs-Löffler bacillus show great pleomorphism of individual bacilli; films from a culture of the bacillus of Hoffmann show bacilli of great uniformity of appearance.

Plate XX shows films from (a) a typical culture of the Klebs-Löffler bacillus, stained with Neisser's stain; and (b) a typical culture of the bacillus of Hoffmann stained by carbol-thionin. For this plate the writer is most grateful to C. M. Hutchinson, Esq., M.A., Imperial Research Institute, Pusa, who very kindly took the photographs, as this Institute unfortunately does not possess the necessary electric current for micro-photographic work.

In the direct films from the swab, differentiation is often difficult, though less so if Ponder's stain be used. In young serum cultures differentiation is easy; and it is also easy to tell a mixed infection. The chief difficulty in cultures indeed is not to differentiate the bacillus of Hoffmann from the bacillus of diphtheria, but to tell the Klebs-Löffler bacillus from certain non-diphtheroid organisms. A field full of cocci stained by Neisser's stain often shows deep brown, not blue, almost contiguous granules. Again, an occasional streptothrix, broken up and showing endogenous granules, may appear at first sight to resemble the Klebs-Löffler bacillus. In all cases, however, Cobbett's blue or Ponder's method will clear the diagnosis.

The real source of confusion in both direct films and cultures is the occasional presence—as the only or as the predominating type of organism—of rod-like bacilli, arranged in typical geometrical patterning and resembling the true Klebs-Löffler bacillus in every respect except for an entire absence of any granules. These are quite unlike the bacillus of Hoffmann with its wide centre, pointed poles, and mesial septum. But they resemble the true Klebs-Löffler bacillus closely and are probably true diphtheria bacilli of a non-beaded type. These are the organisms returned as  $\pm$  in Tables II and III. One such strain (Case 8; swab of 14-10-17) was intensely virulent, 2 c.c. killing a guinea-pig in 19 hours and 1 c.c. in 22 hours.

Having thus defined the terms used, we may pass to a consideration of the circumstances under which the bacillus of Hoffmann was encountered. In January 1919, in the entire absence of diphtheria in the station, in order to obtain some information as to the incidence of the bacillus of Hoffmann in a normal Indian population, the throats of 103 consecutive Indian antirabic patients were examined. One showed the



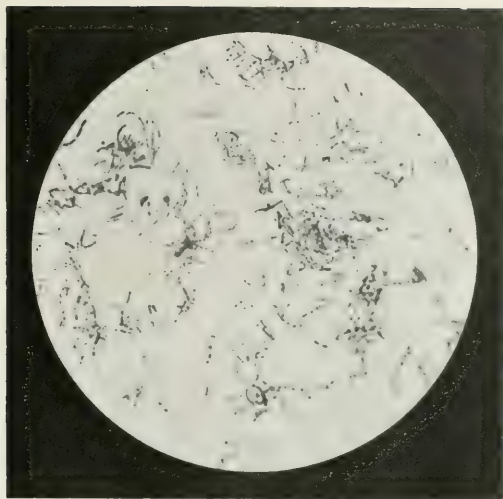


FIG. a.

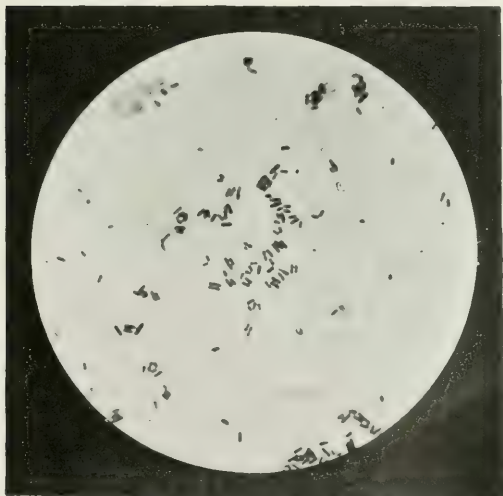


FIG. b.

R. KNOWLES.—The Association of the Bacillus of Hoffmann with Diphtheria in India.





true Klebs-Löffler bacillus, in an apparently normal throat ; and five showed the bacillus of Hoffmann, in only one instance in connection with any accompanying pharyngitis.

Including these observations the bacillus of Hoffmann was met with in the throats of 20 out of 444 individuals examined, 4·5 per cent ; and in 24 out of 623 throat swabs, 3·9 per cent (all examined culturally).

The bacillus of Hoffmann occurred in the following associations :—

*A. In actual cases of diphtheria.*

*Case 12.*—A month after diphtheria, when convalescence was established, and after a finding of non-virulent Klebs-Löffler bacilli.

*Case 14.*—When convalescent, 2 c.c. of a 48-hour broth culture of this organism, however, killed a guinea-pig of weight 300 gms. in 3 days with acute hæmorrhagic œdema at the site of inoculation and acute congestion of the spleen and peritoneum. Cultures on inspissated serum from the animal's peritoneal exudate yielded an organism with pointed poles, non-granular, and a pale mesial septum, *i.e.*, apparently the true bacillus of Hoffmann.

*Case 15.*—Had diphtheria in May 1918. Still showed Klebs-Löffler bacillus (non-virulent) in June 1918. Throat re-examined in February 1919, when the child was in excellent health. The culture yielded very sparse Klebs-Löffler colonies ; and a profusion of growth of the bacillus of Hoffmann. This Hoffmann strain was entirely non-virulent, and with typical sugar reactions—no acidity on glucose broth in 6 days.

*Case 16.*—When convalescence was established ; and preceded and followed by entirely negative findings. Apparently a transient infection.

*Case 18.*—At first pure Klebs-Löffler. Eleven days later, when recovering from the disease, a mixture of both organisms with colonies of Klebs-Löffler predominating. Seven days later a pure culture of the bacillus of Hoffmann. A week later a negative finding.

*Case 19.*—A child with an acute and typical attack, so severe that antidiphtheritic serum was given without awaiting the laboratory report. The culture yielded only the bacillus of Hoffmann. Three injections were given, and a swab 19 days later gave negative results.

## 210 *The Hoffmann Bacillus and Diphtheria in India.*

*Case 22.*—After a finding of Klebs-Löffler during the attack and prior to two negative results.

*Case 24.*—An Indian adult, who presented himself at the Shillong dispensary, 'suffering from fever and with typical membrane over both tonsils, apparently diphtheria.' The swab gave a pure culture of the bacillus of Hoffmann; the strain gave true sugar reactions, and was entirely devoid of virulence. Unfortunately all further attempts to secure a further swab from this most interesting case were unsuccessful.

*Case 29.*—On convalescence from diphtheria in a persistent Klebs-Löffler carrier. At first with the true Klebs-Löffler bacillus; later in pure culture.

*Case 30.*—Found to be a Klebs-Löffler carrier early in the outbreak at the girls' school, 23-7-18. The Klebs-Löffler bacillus was replaced by the bacillus of Hoffmann, 6-8-18 and 12-8-18. The throat then cleared and was negative. On 8-10-18 diphtheria supervened with only typical Klebs-Löffler bacilli in culture.

*Case 36.*—From Gauhati, reported to be clinically diphtheria. The one swab obtained yielded a pure culture of the bacillus of Hoffmann.

The results of examination of 36 cases, of what was clinically diphtheria, were as follows :—

18 showed the typical Klebs-Löffler bacillus, but no other diphtheroid organism.

1 showed  $\pm$  bacilli, followed by typical Klebs-Löffler bacillus.

8 showed both the Klebs-Löffler bacillus and the bacillus of Hoffmann, either simultaneously or at different times.

3 showed the bacillus of Hoffmann, but no other diphtheroid organism.

7 gave entirely negative findings (4 examined only after convalescence).

In diphtheria cases the bacillus of Hoffmann occurred in 11 out of 36 cases, 30 per cent, and in 14 out of 104 swabs, 13 per cent.

### *B. In diphtheria carriers.*

The instances here were as follows :—

*Carrier 12.*—A child living outside either school. Klebs-Löffler on 6-10-17; Hoffmann on 17-10-17.

*Carrier 19* (sister of *Carrier 13*).—Klebs-Löffler on 10-10-17 three days after her brother (a day boy at the school) had shown a similar finding. Hoffmann only, non-virulent, on 18-10-17.

*Carrier 23* (sister of *Case 13*).—An Indian child. Hoffmann, non-virulent, on 14-11-17, when her brother was suffering from diphtheria in the same house. Pure Klebs-Löffler on 26-11-17. The brother proved a persistent carrier on convalescence.

*Carrier 29* at the girls' school.—Klebs-Löffler present in the throat for 39 days. On two occasions out of four the bacillus of Hoffmann was associated with it.

In diphtheria carriers the bacillus of Hoffmann occurred in 4 persons out of 36, 11 per cent; and in 5 out of 101 swabs, 5 per cent.

#### *C. In healthy throats during an epidemic.*

Here there is a most complete contrast to the findings in A and B. The bacillus of Hoffmann was found only in one individual out of 269, 0·4 per cent; and in one swab out of 314, 0·3 per cent. Two hundred and sixty-eight individuals, mostly European children, whose throats were examined as possible contacts with diphtheria cases or carriers, showed no diphtheroid organisms of any type.

Even the one positive finding is not entirely above suspicion, as the child had been to visit the girls' school and was also a close contact of *Case 23*. The strain was entirely non-virulent.

#### *D. In healthy throats apart from any epidemic.*

The throats of a small random sample of 103 consecutive Indian antirabic patients were examined (direct films and cultures) in January 1919. All were Indians. One showed the Klebs-Löffler bacillus in a healthy throat; five, the bacillus of Hoffmann.

#### *The possible relationship of the bacillus of Hoffmann to diphtheria.*

It must be at once admitted that the above findings are entirely at variance with those of other workers in other countries. The evidence

for and against the identity of the Klebs-Löffler bacillus and the bacillus of Hoffmann is well summarised by Besson (1913), Ledingham and Arkwright (1912), and by Nuttall and Graham Smith (1908). Most workers are of definite opinion that the two organisms are entirely different.

Conditions in India, however, are widely different from those in England and temperate climates. In India diphtheria is a disease of limited and sporadic occurrence, and is chiefly met with in European schools in hill stations: widespread epidemics are at least not on record. For some reason, probably meteorological, the disease, when once established, does not tend to spread outside limited communities. Cases met with in general clinical work are sporadic and scattered.

The curious parallelism therefore, in the above figures, of the true Klebs-Löffler bacillus and the bacillus of Hoffmann, may not be without interest. The bacillus of Hoffmann may occur (*a*) before, (*b*) after, or (*c*) simultaneously with the Klebs-Löffler bacillus. In India it is most frequently met with in association with the Klebs-Löffler bacillus and is rather rare apart from diphtheria.

Morphologically, culturally, and as regards pathogenicity, the two organisms are entirely different. But that there may be some symbiotic relationship between the two organisms is probably a view that will accord with the above experimentally observed facts: that diphtheria may develop more easily in a person previously infected with the bacillus of Hoffmann, that the bacillus of Hoffmann may persist more readily in the throat of a diphtheria convalescent than in that of a healthy person: and may also explain the widely divergent findings and opinions of different observers.

In brief the rarity of the bacillus of Hoffmann in India may be associated with the rarity of diphtheria in epidemic form; whilst its very general prevalence in temperate zones may be associated with the general prevalence and frequent epidemics of diphtheria. Whatever the truth, Indian conditions afford an admirable field for the study of this as of other kindred problems. In India cases of such diseases of temperate climates are sporadic only, channels of infection are more evident, and incidence, etiology and relationships perhaps easier to trace than in temperate zones.

## SUMMARY.

1. Both 1917 and 1918 outbreaks showed similar features. Although there was evidence of widespread infection of individuals throughout the station, both epidemics remained very limited. Meteorological conditions in India appear unfavourable to diphtheria epidemics, except during the rains.

2. Both outbreaks appear to have originated from carrier sources; the 1918 one at least certainly did so.

3. The incidence of the bacillus of Hoffmann in India was found to be entirely different from that in temperate climates. It was met with in 30 per cent of cases of diphtheria; in 11 per cent of diphtheria carriers, showing the true Klebs-Löffler bacillus; in only 0·4 per cent of healthy throats among European children during an epidemic; and in only 5 per cent of the throats of 103 Indians in the absence of any epidemic.

4. The bacillus of Hoffmann was met with (a) before, (b) with, and (c) after the presence of the true Klebs-Löffler bacillus in the same throat.

5. Whilst the two organisms appear to be entirely different as regards morphology, cultural reactions and pathogenicity to animals, yet it is suggested that there is a symbiotic relationship between them that the rarity of the bacillus of Hoffmann in India may be associated with the relative rarity of diphtheria in epidemic form. Reverse conditions hold in temperate climates.

TABLE I.  
*Case and Carrier Incidence, 1917-1918.*

			Population concerned.	Cases.	Carriers.
				%	%
Boys' school, 1917	..	..	108	8 = 7·4	14 = 13
Girls' school, 1918	..	..	70	11 = 15·7	6 = 8·6
Outside households, (including Gauhati)	1917-18	..	163	17 = 10·4	16 = 9·2
TOTALS			341	36 = 10·5	36 = 10·5

TABLE II.  
*Findings in Diphtheria Cases, 1917-1918.*

Serial No. of case.	Residence.	Date of appearance of disease.	Findings.	Summary.	REMARKS.
<i>Shillong, 1917.</i>					
1	Boys' school	30-8-17	-1-10-17	-	Examined after convalescence.
2	Do.	12-9-17	-1-10-17	-	Do. Sister of Case 1.
3	Boys' school	20-9-17	-12-10-17	-	Examined after convalescence.
4	Do.	21-9-17	K. L. (Fatal strain) 27-9-17	K. L.	Examined after convalescence
5	Do.	23-9-17	-12-10-17	-	Examined after convalescence
6	Do.	24-9-17	K. L. (Fatal strain) 27-9-17	K. L.	Examined after convalescence
7	Do.	3-10-17	K. L. 3-10-17	K. L.	Examined after convalescence
8	Do.	4-10-17	K. L. 4-10-17	K. L.	Examined after convalescence
9	Do.	8-10-17	18-10-17; 3 non-virulent 14-10-17; K. L. 11-10-17	K. L. ±	Examined after convalescence
10	Do.	14-10-17	K. L. 9-10-17; K. L. 11-10-17; -16-10-17	K. L.	Adult outside school.
11	Do.	16-10-17	K. L. non-virulent 29-10-17; -2-11-17	K. L.	Child outside school.
12	Boys' school	18-10-17	-4-10-17; K. L. non-virulent 14-10-17	-	Adult outside school. Clinically diphtheria.
13	Do.	14-11-17	-6-10-17; 3 virulent 18-10-17; K. L. non-virulent 3-11-17; H. 22-11-17	K. L. H.	Examined after convalescence.
14	Do.	21-11-17	K. L. non-virulent 14-11-17; K. L. non-virulent 18-11-17; K. L. 21-11-17; K. L. non-virulent 26-11-17; -30-11-17	K. L.	Indian child.
15	Do.	8-5-18	K. L. 21-11-17; -26-11-17; H. virulent; 28-11-17	K. L. H.	Indian child.
<i>Shillong, 1918.</i>					
16	Do.	8-5-18	K. L. 10-5-18; K. L. 18-5-18; -24-5-18	K. L.	Child outside school. Came from Gauhati.
17	Do.	19-2-18	K. L. 31-5-18; K. L. non-virulent 8-6-18	K. L. H.	Child outside school. Came from Gauhati.



16	Girls' school	14-7-18	K. L. 14-7-18; K. L. 30-7-18; -6-8-18; H. 13-8-18; -31-8-18; -1-9-18; -8-10-18	K. L. H. K. L.	....
17	Do.	16-7-18	K. L. 17-7-18; -30-7-18; -8-10-18	K. L. H.	....
18	Do.	19-7-18	-17-7-18; K. L. 19-7-18; K. L. H. 30-7-18; H. 6-8-18; -12-8-18	K. L. H.	....
19	Do.	20-7-18	H. 20-7-18; -8-10-18	—	Clinically diphtheria.
20	Do.	20-7-18	-20-7-18; -12-8-18; -13-8-18; -14-8-18; -8-10-18	—	Clinically diphtheria.
21	Do.	23-7-18	-20-7-18; K. L. 23-7-18; -12-8-18; -19-8-18; -8-10-18	K. L.	....
22	Do.	26-7-18	K. L. 26-7-18; H. 12-8-18; -19-8-18; -8-10-18	K. L. H.	....
23	....	1-8-18	K. L. 1-8-18; K. L. non-virulent 14-8-18; -22-8-18	K. L.	Adult outside school.
24	....	5-8-18	H. non-virulent 5-8-18	H.	Clinically diphtheria.
25	....	25-8-18	K. L. 26-8-18	K. L.	Indian. Mother of Case 26.
26	....	26-8-18	K. L. 28-8-18	K. L.	Indian. Son of Case 25.
27	Girls' school	29-8-18	K. L. 29-8-18; -20-9-18; -26-9-18	K. L.	Mother of Case 16 and visited her.
28	Do.	3-10-18	-19-7-18; K. L. 3-10-18; -9-11-18; -15-11-18	K. L.	....
29	Do.	5-10-18	-17-7-18; -20-7-18; K. L. 5-10-18; K. L. H. 8-11-18; H. 15-11-18	K. L. H.	....
30	Do.	8-10-18	-22-11-18 -20-7-18; K. L. 23-7-18; H. 6-8-18; H. 12-8-18; -19-8-18; K. L. 8-10-18; -20-10-18; -24-10-18	K. L. H.	Carrier first; then case.
<i>Gauhati, 1918.</i>					
31	....	13-6-18	—	—	Indian. Clinically diphtheria.
32	....	13-6-18	K. L. 13-6-18	K. L.	Indian. Brother of Case 31.
33	....	27-6-18	K. L. 26-7-18	K. L.	Indian.
34	....	5-7-18	K. L. 5-7-18	K. L.	Indian.
35	....	14-8-18	K. L. 14-8-18	K. L.	Indian. Clinically diphtheria.
36	....	7-10-18	H. 7-10-18	H.	Indian.

TABLE III.  
*Findings in Diphtheria Carriers, 1917-1918.*

Serial No. of Case.	Residence.	Findings.	Summary.	REMARKS.
<i>Shillong, 1917.</i>				
1	Boys' school ..	K. L. 30-9-17 : -9-10-17 ..	K. L.	....
2	Do. ....	K. L. 3-10-17 : -12-10-17 ..	K. L.	....
3	Boys' school ..	-4-10-17 : K. L. non-virulent	K. L.	Child outside school.
4	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
5	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	Sister of Case 10.
6	Boys' school ..	-4-10-17 : K. L. non-virulent	K. L.	....
7	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
8	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
9	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
10	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
11	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
12	Boys' school ..	-4-10-17 : K. L. non-virulent	K. L.	Child outside school.
13	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
14	Boys' school ..	-4-10-17 : K. L. non-virulent	K. L.	Child outside school.
15	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
16	Boys' school ..	-4-10-17 : K. L. non-virulent	K. L.	Young baby outside school.
17	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
18	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
19	Boys' school ..	-4-10-17 : K. L. non-virulent	K. L.	Sister of Carrier 13.
20	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	....
21	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	Sister of Carrier 22.
22	Do. ....	-4-10-17 : K. L. non-virulent	K. L.	Sister of Carrier 21.
23	Do. ....	-4-10-17 : K. L. non-virulent	H. K. L.	Sister of Case 13.

## Shillong, 1918.

24	Girls' school	K. L. 4-2-18; K. L. non-virulent 12-2-18; -19-2-18	K. L.	Contact of Calcutta Case, Jan. 1918.
25	....	K. L. 17-7-18; -30-7-18; -6-8-18; K. L. 8-9-18; K. L. 20-9-18; K. L. 26-9-18; -3-10-18; -8-10-18	K. L.	....
26	....	K. L. 18-7-18; K. L. 27-7-18; K. L. 3-8-18; -10-8-18; -17-8-18	K. L.	....
27	Girls' school	K. L. 18-7-18; -6-8-18; -10-8-18; -17-8-18	K. L.	Father of Gauhati Case, June 1918.
28	Do.	K. L. 19-7-18; -30-7-18; -6-8-18	K. L.	Sister of Gauhati Case, June 1918.
29	Do.	K. L. 19-7-18; -30-7-18; K. L. H. 6-8-18; K. L. 12-8-18; -19-8-18; K. L. H. 27-8-18; -5-9-18; -12-9-18; -8-10-18; -26-2-19	K. L.	....
30	Do.	K. L. 20-7-18; K. L. 30-7-18; K. L. 6-8-18; -12-8-18; -19-8-18; -8-10-18	K. L.	....
31	Do	-17-7-18; -19-7-18; K. L. 14-8-18; K. L. 23-8-18; -5-9-18; -12-9-18; -8-10-18	K. L.	....
32	Do.	-18-7-18; -27-7-18; K. L. 5-10-18; -20-10-18; -24-10-18	K. L.	....
33	....	K. L. 7-10-18; -8-10-18	K. L.	Daughter of Carrier 26.
34	....	K. L. 10-10-18; -12-10-18	K. L.	Adult outside school. Child outside school.
35	....		K. L.	Contact of Case 32.
36	....		K. L.	Contact of Case 32.

## Gauhati, 1918.

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## OBSERVATIONS ON THE CULTURAL METHODS OF GONOCOCCUS.

BY

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GONORRHOEA is a very wide spread disease, and the causative organism being strictly parasitic on the human race, its harmful and far-reaching effects cannot be over-estimated. Although the causative organism was recognised and isolated as far back as 1879, it has practically resisted a really successful cultivation for the last four decades, as would appear from the vast literature that has sprung up in this direction and the elaborate media planned and worked out for its growth.

In the West the question was taken up long ago, and as the result of that, various brands of gonococcus vaccine are now-a-days available on the market. In India it was never taken up earnestly until quite recently, after the experience in the late war. It is from the venereal war hospital in this brigade that I got suitable cases to carry on my investigations.

This being a field laboratory attached to a general hospital, where routine pathological work for the whole brigade had to be carried out, facilities for taking up elaborate researches were not available, such as are necessary to estimate the comparative value of various complicated media suggested for the growth of this refractory organism. Consequently the plan was to avoid the complicated and use the simple, ordinary media for its cultures.

The following media were selected for test :—

1. Blood (human) agar.
2. Loeffler's blood serum.

3. Legumin agar.
4. Nasgar (Mervyn Gordon).
5. Ordinary agar smeared with human blood.
6. Nutrose agar (Mackenzie Wallis)(1).

Cultures were taken with ordinary precautions from acute cases of gonorrhœa showing only typical gram-negative diplococci both free and intracellular in the smear from the urethral discharge. Tubes were incubated under ordinary conditions for three consecutive days and results noted after that time. The experiment was repeated during several months, the results being invariably negative in cases of gonococci and only partially successful in cases of meningococci.

Since the paper published by Charles W. Chapin(2) in the *Journal of Infectious Diseases*, both meningococci and gonococci have been cultivated under reduced oxygen tension on the following media :—

1. Loeffler's blood serum.
2. Urine-agar with 5 per cent egg-yolk.

The process is very simple and consists merely in generating CO<sub>2</sub> by the interaction of sodii bicarbonas and dilute sulphuric acid in an air-tight jar in which inoculated tubes have been placed before. Details of the procedure will be dealt with in a subsequent paper, as observations on this line had already been initiated by Lieut.-Colonel E. D. W. Greig, C.I.E., I.M.S., in connection with the cultivation of meningococcus before he left for Europe.

Primary cultures from acute cases are successful on both the media, provided a fair quantity of purulent exudate is used during planting. In a tube of 18 to 20 hours' growth, organisms microscopically typical, biscuit-shaped gram-negative diplococci, appear on Loeffler's serum, but are somewhat swollen and round on urine-agar. In a 24 hours' growth the organisms begin to undergo lysis, and many giant forms are found either in pairs or in tetrads. In the following experiments I propose to give the comparative value of these two media as to the maximum yield of bacterial growth obtained in each case, planted either in the ordinary way or coupled with reinforcing processes. The following procedure was strictly observed in each experiment :—

- (1) Tubes used in each case were of the same size ; that is to say, they afforded the same area of sowing surface.
- (2) The same loopful of 18-hour culture of gonococcus on Loeffler's serum was planted in each tube.

- (3) Condensation water at the bottom of the tube was pipetted off during planting. This is done to ensure that it may not contribute to the opacity of the final emulsion for standardizing the growth.
- (4) All tubes were incubated and tested for sterility before planting.
- (5) Culture tubes were incubated at 37°C. for 18 hours in an atmosphere containing 10 per cent CO<sub>2</sub> and sufficient moisture.
- (6) Eighteen hours' growth in each tube was emulsified in 1 c.c. of sterile 0.85 per cent NaCl solution and standardized by the opacity method.

*Experiment 1.*—Lœffler's blood serum planted, and growth emulsified after usual time of incubation.

Opacity of the emulsion = 1800 millions per c.c.

*Experiment 2.*—Culture tube the same as in Experiment 1, but the inoculated surface flooded with a few drops of *fresh, sterile sheep's serum*. Growth emulsified as before.

Opacity of the emulsion = 2400 millions per c.c.

*Experiment 3.*—Urine-agar slope with egg-yolk planted, incubated, and the growth emulsified as before.

Opacity of the emulsion = 1700 millions per c.c.

*Experiment 4.*—The same as Experiment 3, but the inoculated surface was flooded with *fresh, sterile sheep's serum*. Growth emulsified as usual.

Opacity of the emulsion = 1700 millions per c.c.

*Note.*—In Experiments 3 and 4, some opacity is due to egg-yolk going into solution during emulsification. So the results expressed in terms of bacterial opacity are not strictly accurate. For the same reason they are neither strictly comparable with each other nor with those of Experiments 1 and 2. A film preparation from each emulsion, however, shows a more luxuriant growth in the tube to which fresh serum was added.

*Experiment 5.*—Lœffler's serum planted, and the surface flooded with a few drops of *heated, sterile human serum*. Growth emulsified as usual.

Opacity of the emulsion = 2100 millions per c.c.



*Experiment 6.*—The same as in Experiment 5, but instead of *heated human serum*, *fresh sterile human serum* was used.

Opacity of the emulsion = 2700 millions per c.c.

#### CONCLUSIONS.

1. *Gonococcus* is best cultivated under reduced oxygen tension.
2. Primary cultures grow equally well on Loeffler's blood serum or urine-agar with egg-yolk, provided a fair amount of purulent exudate is used.
3. Sub-cultures grow better on Loeffler's blood serum.
4. Bacterial yield can be augmented by adding *fresh serum*, preferably *human serum*.
5. Human serum heated to destroy its bactericidal properties gives no advantage over *fresh serum* in promoting growth of *gonococcus*: probably the vitamine content is destroyed to some extent by heating.

My best thanks are due to Lt.-Col. W. E. McKechnie, I.M.S., Officer Commanding, No. 37 Indian General Hospital, for affording every available facility to take up research work in the laboratory which is attached to his unit. My thanks are also due to Major James, I.M.S., Officer Commanding, Venereal Laboratory War Hospital, for selecting suitable cases for my observations.

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## ‘STUDIES IN ANKYLOSTOMIASIS’ : A CRITICISM.

BY

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[Received for publication, May 15, 1919.]

As the result of his ‘Studies in Ankylostomiasis’ (5) Captain Wrench has reached the following conclusions :—‘ Much work and money are now being spent, and it is proposed to spend more, upon organisations for the cure of ankylostomiasis by chenopodium oil in particular, and secondarily by thymol, on a very extensive scale. In our opinion this effort cannot attain the object which it presumes it can.’ Elsewhere cure is defined thus :—‘ What constitutes a cure ? The orthodox answer is, the complete destruction of all ankylostome worms in the body.’

Since the allotment of funds for future hookworm work will lie so largely in the hands of lay administrators, it seems most essential that it should be widely realised that Captain Wrench’s conclusions, even if accepted to the full, do not in any way alter the hookworm question which confronts the administrator at the moment. It is immaterial from this point of view whether former cures were complete or incomplete. It is material that these very cures have resulted, both in and out of India, in a marked benefit to the health and earning capacity of those previously infected. The money now being allotted for a similar purpose is being put aside in order that other infected persons may receive similar benefits by similar methods under similar conditions. To delay this work on the ground that investigation will produce, and until investigation has produced, a perfect or more nearly perfect line of treatment is surely not only economically unsound but morally unjustifiable.

But if the administrator is thus morally compelled to attempt to obtain without delay for the many the benefits which have already been obtained for the few, regardless of the results of these investigations, it is quite otherwise with the helminthologist, the medical man and the sanitarian. For them this work brings out in an acute form the urgent necessity of meeting the three great needs of the hookworm question which are outstanding at the present time.

These are—(1) the devising and use of suitable latrines, (2) the improvement in accuracy and rapidity of diagnostic methods, and (3) the finding of an anthelmintic which will safely disinfect in a single dose.

Regarding the first, it may be noted that the paper under consideration appears to offer strong presumption that reinfection was taking place during the course of the work. There are, for example, four instances in which ova reappeared in the stools 44, 45, 47, and 51 days after the last treatment, that is to say just at that interval of time which corresponds to the observed interval between hookworm infection and hookworm maturation. In view of these suggestive cases it is unreasonable to be asked to accept without proof the statement therein made that 'as our hospital is situated in an arid desert reinfection by the recognised methods was impossible.'

No evidence is offered for the correctness of this statement. There is for example no estimate of the degree of infection of the native inhabitants of the part, no mention of the kind, and habitual aridity, of latrines used in the hospital, and the consequent possibility of infection there. The statement must surely be considered as a personal opinion: one indeed which the facts of the case do not appear to justify.

Latrines are rarely arid spots and the question of acquiring infection from latrines is one attaining great general importance at the present time, when certain types of these are perforce being recommended for rural use, despite the fact that a satisfactory self-acting latrine is, notwithstanding hopes and statements to the contrary, still unknown, or at least unproved.

So important from the sanitarian's standpoint is the possibility of infection being acquired at the time of defecation that it seems desirable to emphasise such infection by the use of the special term 'dejectional,' whether the spot of acquisition be a latrine or merely some favoured and sequestered place visited regularly for purposes of nature; whereas other infection may be called 'casual.' In Ceylon (4), in gardens on

which latrines had been erected prior to treatment, re-examinations made one, three, and six months later showed that 20, 27·3, and 31·3 per cent respectively of those formerly found free from infection were again infected. The very gradation of the figures affords cogent evidence of reinfection. While admitting that the conditions described in that report do not admit of a conclusion as to whether this reinfection was dejectional or casual, the possibility of the former must, under the circumstances, be at least considered. It seems in this connection fair to urge that no latrine can be considered as satisfactory which is not demonstrably ovicidal or larvicidal under all conditions of reasonable usage.

It appearing fair to conclude that reinfection, dejectional or casual, was occurring during Captain Wrench's observations, this conclusion naturally affects their application to the general problems of diagnosis and treatment.

Regarding improvement of diagnostic methods in accuracy and rapidity, his figures seem to indicate the practical inadequacy in both these respects of the methods which he employed. In the matter of accuracy, for instance, variations from day to day in what is presumed by him to be the same quantity of stool are as follows :—

*In 45 cases in Chart I.*

Serial number.	Variations in number of eggs.	REMARKS.
10	Fifty-fold .. ..	Before treatment.
15	Sixteen-fold .. ..	Do.
29	Twenty-seven-fold .. ..	Do.
30	Twenty-five-fold .. ..	Do.
32	Fourteen-fold .. ..	Do.
38	0 to 51 .. ..	After third treatment.
40	Fifteen-fold .. ..	Before treatment.
41	Fifteen-fold .. ..	Do.
42	Seventeen-fold .. ..	Do.
43	Forty-fold .. ..	Do.
43	Twenty-four-fold .. ..	After second treatment.

*In 19 Cases in Chart II, no examinations being made before  
treatment.*

Serial number.	Variations in number of eggs.	REMARKS.
6	Twenty-fold .. ..	After third treatment.
7	Thirteen-fold .. ..	Do.
9	Twelve-fold .. ..	After first treatment.
13	Twenty-fold .. ..	.. second ..
13	Sixteen-fold .. ..	.. sixth ..
15	One-hundred-and-twenty-fold .. ..	.. first ..
15	Thirteen-fold .. ..	.. second ..
19	Sixty-four-fold .. ..	.. second ..
19	Fourteen-fold .. ..	.. third ..
19	Seventeen-fold .. ..	.. sixth ..

While certain variations occur from day to day in all infected stools, it is my experience that recurring variations of this extent do not occur under these conditions in the same person's stool, if accurate steps be taken to insure that the same quantity of stool shall be used for each examination. In the matter of rapidity, the number of examinations estimated by Captain Wrench as requisite to establish a negative diagnosis by his technique seems to put it out of the range of practical usage.

It may incidentally be noted that his statement that the most complete examinations by the workers of the Rockefeller Foundation are those in Trinidad is unintentionally unfair, since for instance the minimal routine examination in Ceylon in 1916-17 (4) consisted of two uncentrifuged and three centrifuged slides before a specimen was pronounced to be negative. The fact is that growing experience has independently convinced workers in different parts of the world that the examination of a single centrifuged slide is insufficient for an accurate opinion. It follows, as Captain Wrench implies, that the Darjeeling figures are inaccurate, although the diagnostic technique was adopted from Howard's description and chosen as being apparently the most accurate available at the time. It must be added that it was a similar



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## THE TECHNIQUE OF THE LEVITATION METHOD.

BY

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IT having been pointed out that the description previously(1) given of the Levitation Method for the concentration of hookworm eggs was inadequate for one who has never seen it used, the following full description is given. It is possible that, just as the former description has proved too short, this may be too long, but the difficulties of adequately condensing a description of a technique of this character are great, and it is thought best to be complete at the possible risk of being prolix. The description is aided by the republication of the diagrams used to illustrate the former paper.

Since Levitation consists of an addition to the ordinarily used Centrifugal Method of concentration for hookworm eggs, its requisites are divisible into two categories: first, those necessary for the concentration of ova by the centrifuge, a method which, apart from the modifications designed to ensure accurate measurements at all stages of the process, was, I believe, first described by Dr. H. H. Howard; and second, those necessary for the further removal of camouflage by the washing of the centrifuged faeces in a particular manner. It may be added that the Levitation Method has given consistent results in the hands of a number of different persons, and that the failure to obtain them in any instance is more likely to be due to defect in technique than to any defect in the method itself. The syringe measure, sliders and depressor may now be purchased from Smith Stanistreet & Co., Calcutta.

For the first category of requisites just mentioned, the apparatus required is as follows:—

1. A half-cubic-centimeter measure capable of being accurately worked by a sweeper and apparently foolproof (Figs. 10 to 13). This

may be said to consist practically of the barrel and piston of a hypodermic syringe without a nozzle and so made that the piston will slip right through the barrel, while being so much shorter than the barrel that the space left unfilled between the two measures exactly half a c.c. If real accuracy be required, I believe that this apparatus can be depended upon to give it in the hands of a sweeper. If only an approximate measurement be necessary, as would be the case in diagnostic work on a large scale, it would suffice to issue phials with a mark indicating, say 5 c.c., and placing in them  $4\frac{1}{2}$  c.c. of a preservative, the sweeper adding faeces till the surface of the fluid reached the indicating mark, as is done at the Parel Laboratory, Bombay.

2. Two test-tubes of fairly wide calibre, and having the usual lip, one of them being fitted with a cork.

3. A supply of wire gauze, having 100 meshes to the linear inch.

4. A centrifuge.

5. Centrifuge tubes consisting of a number of pieces of glass tubing of such diameter and cut into such lengths as will, when corked, suit the centrifuge in which they will actually be used—ordinarily they should be as large as possible.

6. Corks to fit these centrifuge tubes.

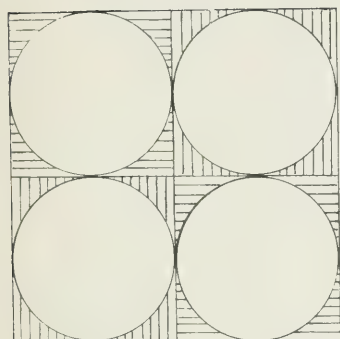
7. A pair of dissecting forceps.

The technique is as follows:—The sweeper places the syringe measure vertically on a flat surface, sees that the piston as well as the barrel are touching that surface, fills the cavity with faeces from several parts of the stool by means of a sliver of wood, and with this scrapes off the superfluous stool level with the edge of the barrel. By pushing the piston out from below, the contained stool is ejected into a wide-mouthed test-tube. Sufficient water is added to fill one of the centrifuge tubes about three-quarters full; the test-tube is corked and is energetically shaken by the sweeper for a minute, which should suffice to break up the stool completely.

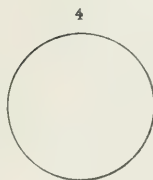
If the centrifuge tubes be of sufficient width a piece of gauze is engaged in the mouth of one of them and the faecal suspension strained into it. If the centrifuge tube has perforce to be narrow the straining must be into the second test-tube, the strained fluid being immediately transferred to a centrifuge tube.

If the centrifuge be a 'fly-out' one, no corks are required in the upper end of the tubes; if the tubes have to be put in horizontally or nearly so, such a cork is obviously necessary. The tube is then

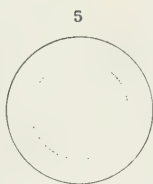
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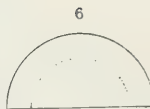
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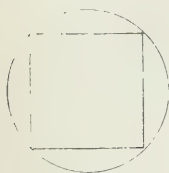
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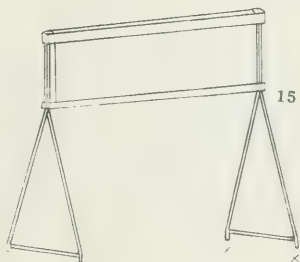
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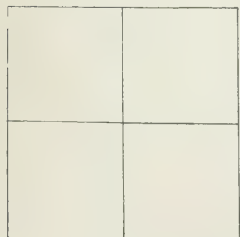
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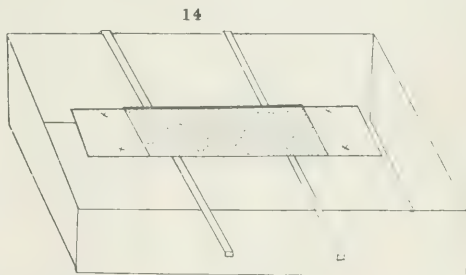
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14

CLAYTON LANE.—The Technique of the Levitation Method.



centrifuged at such rate and for such time as actual tests show to be necessary to bring down all ova in that particular centrifuge as a deposit, this deposit together with much faecal matter collecting on the face of the cork. It is the separation of the ova and faecal matter contained in this deposit which is the aim of all that here follows.

For the second category, that is for the proper performance of concentration by levitation, the following apparatus is necessary :—

8. A pair of fairly rigid yet flexible, thin, resilient, smooth, flat iron bars, with the ends bent at right angles for about  $\frac{1}{4}$ th inch, and about an inch longer than the width of the vessel across which they will presently lie. They are referred to below as 'sliders.'

They must be fairly rigid, in order to bear without bending the weight of the slide together with the overlying meniscus; flexible, because the slide when lying on them must be pushed under water and completely immersed; thin, because the resistance to such immersion must be slight to avoid interference with nicety of subsequent movement; smooth, so that the slide may glide over them without jerking, since this is likely to jar the ova off the glass into the overlying fluid; longer than the width of the vessel, so that they may permit of the slide being deeply immersed without this depression being checked by the engagement of both turned-down ends with the edges of the vessel; flat, so that they shall not roll, in which case the turned-over ends would not necessarily be pointing downwards, and would not prevent the whole apparatus from falling into the water, as the rods shifted position during depression.

9. A depressor, an arrangement for depressing the slide and moving it along the sliders until it emerges wholly from the water. This consists of two rods with turned-down ends and having one and an eighth inches clearance between the ends, fixed rigidly together at a distance of two and a half inches by other rods in such a way that the connections are comfortable to hold and are not immersed with the slide.

The clearance of the ends of the rods must be one and an eighth inches so that when they are brought down over it they shall not touch and jar the slide as the depressor is lowered into place; they are separated by two and a half inches so that they shall neither touch the meniscus nor slip over the end of the slide during immersion; the ends must be turned over so as to engage and move the slide along the sliders.

10. An oblong dish; an enamelled soap dish serving the purpose.

11. A pipette; a fountain pen filler sufficing.

12. A means of limiting the meniscus, such as a grease pencil, or vaseline on a sliver of wood.

13. A stirrer ; a sliver of wood sufficing.

14. A folded duster.

The description of the means of separating, by the aid of this apparatus, the ova and faecal matter is as follows :—

The area which the meniscus will cover is first limited by drawing on the slide with a grease pencil or vaseline the limits of the field of observation afforded by the particular mechanical stage used(1). The depressor described above allows of this being two inches long, the usual  $3 \times 1$  inch slide being employed. The soap dish is filled about two-thirds full of water and the sliders placed across it parallel to one another and about one and a half inches apart. Across the sliders is placed the slide and a few drops of water from a pipette collected on the middle of this. The tube containing the strained and centrifuged stool is taken, the upper cork removed, if there has been one there, and the fluid poured away from the upper end, care being taken that none of the sediment passes with it. Holding the tube over the center of the slide, and tilting it so that it is oblique, the cork is first freely rotated within the tube, a manoeuvre which insures that most of the deposit will leave the tube with the cork, and then withdrawn lower edge first, so that the fluid and much of the sediment will run on to the middle of the slide without soiling the fingers. The cork is stood, small and dirty end downwards, on the slide while a few drops of water from the pipette are run through the tube, the rotation of which aided possibly by a touch or two from a sliver of wood passed through the upper end will carry all the remaining deposit on to the slide. The centrifuge tube is then discarded and the small dirty face of the cork smeared gently on the slide, where the water lies on this, and then lifted and washed clean by running over it a few drops of water from the pipette, which carry on to the slide the remains of the deposit, after which the cork is discarded in turn. Water is then added from the pipette till there lies on the slide a considerable meniscus,\* limited by the edges of the slide and by the grease lines drawn across it. With a sliver of wood the deposit is thoroughly stirred up within the meniscus sufficiently energetically to break it up completely so that no conglomerations of deposit are visible, but not sufficiently

\* The slide must be quite horizontal for this purpose, a condition effected either by putting a sliver of wood under the dish, at the required end ; or, as Dr. G. W. Thompson has pointed out, by standing the dish in a sand-bath.

energetically to make it spill. The slide is then left undisturbed for five minutes so that any contained eggs may sink to and come to rest upon the glass. One of the reasons for using a wire gauze with at least 100 meshes to the linear inch, is that it insures that there shall lie within the meniscus few solid particles larger than a hookworm egg. These eggs will accordingly be among the first particles to reach the glass by gravity, and will not be crowded off it by the previous arrival thereon of yet larger particles, nor will they be brushed off it later by the bumping into them of such large particles. It cannot be too strongly urged that unless the eggs lie on the glass when washing begins, they cannot possibly be found upon it when the washing is over, and that strict attention to detail is necessary if success is to be obtained. It is not easy to define in description the proper size of the meniscus. If too deep it will spill, but, short of that, depth is beneficial. The optimum depth is soon learnt by experience.

The meniscus having stood for five minutes the immersion of the slide is carried out as follows :—

The depressor being taken between the thumbs and first two fingers of both hands, and these being steadied by resting the tips of the little fingers on the ends of the dish, the depressor is brought down gently so that the depressing rods lie on the slide one on each side of the meniscus. It is then pressed gently downwards carrying with it the slide on the flexible sliders. It is, I now think, best to keep the slide quite level during depression, and to continue the movement evenly and not too rapidly, directly downwards until the meniscus and the water in the dish commingle and the slide with nearly all the deposit still lying on it is well below the surface. The optimum rate of immersion is one which neither allows of the deposit spreading through the water in the dish, nor of the water in the dish forcing the deposit into a 'cock's comb' along the midline of the broken meniscus. The slide is then drawn along the smooth sliders towards the operator, until it begins to emerge from the water, as it must when it reaches that part of the sliders which is not immersed. As it emerges, the deposit is combed off it by the surface tension and is left behind in the water, while the slide become almost free from visible solid matter, particularly if it has been withdrawn somewhat obliquely so that the last part to emerge from the water is one corner and not the whole length of the further edge. If dissatisfied with the clarity of the slide a second meniscus should be formed by running (not dropping) water very gently over it from a



pipette, and immersing again in the same manner as before. It is from actual counting before and after such a second washing, in cases in which the first washing has not been wholly satisfactory in cleansing, that some of the strongest evidence on the effects of camouflage in preventing accuracy is obtained. If, however, instead of forming a second meniscus the slide be merely lowered into the water a second time and then withdrawn, it is my experience that eggs will be washed away and success not obtained.

The cleaned slide is lifted from the sliders with a pair of forceps, the under surface wiped on a duster, the slide placed on the stage of the microscope, and from the pipette enough water run gently upon it to form a definite meniscus, the object of which is to prevent any possibility of drying during examination, while the slide itself will be so clear that there does not lie upon it the material to form a surface layer of solid matter camouflaging the underlying ova, as is the case if a large quantity of water be used to dilute an ordinary faecal film.

If too much water be used to form this meniscus, the refraction at the curved surface, particularly along the four edges, will interfere unduly with the flatness of the field.

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## A NOTE ON THE PRESENCE OF ACID-FAST BACILLI IN THE BLOOD OF LEPERS.

BY

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Numerous observers have recorded the presence of acid-fast bacilli in the circulating blood of lepers. Honeij (1915) gives a summary of the findings of different workers and states that he himself has found acid-fast bacilli in the blood of 8 out of the 16 cases which he examined. The opinions of the various workers whom he quotes are not, however, unanimous. Some, though they have seen the bacilli in blood films, have considered that they came actually from the tissues and not from the circulating blood; others have failed to find the bacilli at all. Many of those who have recorded positive findings are confident that the bacilli were actually present in the circulating blood.

I have examined the blood of 40 cases of undoubted leprosy, all inmates of the Leper Asylum at Sabathu in the Punjab, and also two other cases suspected of leprosy sent for diagnosis. The blood of 14 healthy persons was examined at the same time as a control on the method of examination.

The technique employed throughout is the same as that employed by Rivan and Smith (1914) and is as follows:—

1 c.c. of blood is drawn off from the median basilic vein at the bend of the elbow and added to 10 c.c. of a 2 per cent solution of acetic acid—this solution having previously been passed through a porcelain filter. The mixture is shaken and then centrifuged for 15 minutes. Two slides are made from the sediment. The films after drying and

fixing in the flame are steamed with carbol fuschin for 5 minutes, decolorised with 5 per cent sulphuric acid for varying lengths of time, according to the thickness of the smear, washed thoroughly and counterstained with Loeffler's methylene blue.

In no case was there any visible leprous lesion at the site of venepuncture. All the slides were first thoroughly searched by me for acid-fast bacilli morphologically conforming to the type of lepra bacilli and then by Sub-Assistant Surgeon Nand Lal, to whom I am deeply indebted for the trouble taken in the examination. In no case was a slide considered positive unless bacilli were found in many fields. Out of the 40 cases of undoubted leprosy, 4 out of 10 cases of the nodular type, 1 out of 20 of the anæsthetic type, and 2 out of 10 of the mixed type, gave a positive result, as shown in the accompanying table. The 2 cases sent for diagnosis with nodular lesions were specially interesting as neither showed leprosy bacilli in these lesions. One of them, however, showed acid-fast bacilli in the nasal mucus and in blood preparations, while the other showed these only in the blood preparation. The bacilli were seen lying free in the blood films and also in mononuclear leucocytes and were usually found in clumps. The blood films of the 14 healthy persons used as controls showed no acid-fast bacilli.

*Table showing results of the examination of blood for the presence of acid-fast bacilli, agreeing morphologically with leprosy bacilli.*

Type of disease.	Number examined.	Number positive.	Percentage of positive result.
Nodular	10	4	..
Anæsthetic	20	1	..
Mixed	10	2	..
TOTAL	40	7	17.5

#### CONCLUSIONS.

1. Acid-fast bacilli agreeing morphologically with the leprosy bacillus are demonstrable in a considerable proportion of blood films made from lepers. The presumption is that these are present in the blood itself, although there remains the possibility of their having been

derived in the process of venepuncture. The skin however at the site of puncture showed no sign of leprous affection. The possibility of the presence of acid-fast bacilli in the reagents used has been excluded by the use of the blood of healthy persons as controls.

2. The examination of the blood may be used with advantage as a routine method of diagnosis in cases of suspected leprosy. It is possible that this examination may sometimes afford evidence of the affection when the ordinary method of examination has failed.

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# FURTHER OBSERVATIONS ON THE STANDARDIZATION OF BACTERIAL SUSPENSIONS.

BY

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## I. DISCREPANCY IN THE RESULTS OBTAINED WHEN USING DIFFERENT RECOGNISED METHODS.

THE three most generally used methods for estimating the number of organisms in a bacterial suspension are :—

1. Wright's method (1902).
2. The hæmocytometer method.
3. The method of colony count.

Glynn, Powell, Rees, and Cox (1913) have maintained that, when the results of standardization of a vaccine by these three methods are compared, the count by Wright's method and by plate culture are both consistently less than that given by the hæmocytometer, in the first case up to 200 per cent less, and in the second up to 60 per cent less.

Similarly, although the figures given by Wilson and Dickson (1912) are almost identical with those found by Brown and Kirwan (1915) as representing the number of *Staphylococcus aureus* in 1 mgm. of dried bacterial substance, their estimations of the numerical content of 1 mgm. (dry) of the several members of the typho-coli group differ markedly from ours.

Wilson and Dickson's findings in this respect were based on Wright's count throughout, whereas our figures for the typho-coli organisms were arrived at by using a combination of the gravimetric method and

that suggested by Braxton Hicks (1912). The results obtained by us are shown in Table I.

## II. THE HÆMOCYTOMETER METHOD.

The standardization of bacterial suspensions by means of the hæmocytometer was suggested by Mallory and Homer Wright (1908): they used a shallow well of 0.02 mm. depth with an optically plane coverslip, a high power dry objective, and no staining fluid.

Since then numerous modifications in technique have been introduced, more especially as regards the nature of the diluting fluid used.

Glynn, Powell, Rees and Cox (1913) advocate the use of a dilute carbol-thionin solution and state that, either an ordinary Thoma-Zeiss hæmocytometer with a well 0.1 mm. deep, or one with a well 0.02 mm. deep, may be used. They conclude that the latter gives little more consistent results than the former and are of opinion that this procedure is the most accurate one which we possess for the numerical estimation of bacterial suspensions.

In the trials I have made of this method I used, in the first instance, the following technique, which was already in use for the purpose and was suggested to me.

The suspension was mixed with an equal volume of freshly filtered Ziehl-Neelsen's carbol-fuchsin and the further dilutions were made with distilled water.

With this technique I have met with astonishing results. In my hands bacterial suspensions of some organisms gave rise to no anomalous results when stained and diluted in this manner. But on the other hand certain suspensions of organisms did not give the results which they should have done and I was at a loss to account for the source of the difficulty. Then I discovered that with two different strains of *B. typhosus* in my possession I obtained widely differing results with each. The suspension from one of these strains, when stained, diluted, and put up for counting gave an absolutely blank field. The bacilli had disappeared completely. This occurred not once or twice but invariably, and the difference between this and the other strain which gave ordinary counts persisted as a characteristic and distinguishing feature. It was obvious then that, even with the hæmocytometer method of standardization, very varying counts could be obtained, varying, not only according to the reagents used, but according to the strain used.

I fully convinced myself that the disappearance of bacilli in the case of one of the divergent strains was not due to agglutinated bacilli which had adhered to the lower surface of the cover glass, and no clumps of bacilli were to be seen in the preparation. What the exact reason for the phenomenon was, whether complete agglutination by the staining fluid, disintegration by the staining fluid, or some other cause, I do not know. One thing however is certain and that is that it was not due to the reaction of the medium. The fact remained that whereas one strain of the same organism regularly gave satisfactory results, the bacteria of the other strain, when treated in precisely the same way, almost entirely disappeared by the time they reached the counting chamber.

This very striking difference in the behaviour of these two strains served to explain certain anomalous and unexpected results obtained with other organisms in the course of my investigations.

These investigations were originally intended to be a revision of the tables of numerical values originally given by Kirwan and myself. The revision was not a *correction* of the values but a replacement by values obtained by an entirely different technique. Incidentally they served to show that numerical values for bacterial suspensions varied enormously with the particular technique used, and not only so, but even with slight variations of the same technique. They have led me, as will be evident from what follows, to the conclusion that all numerical values, by whatever method obtained, are conditioned by the technique used and the details of that technique.

I have shown that the opacity of bacterial suspensions bears a very close and constant relation to the weight of *dried* bacterial substance contained. It seems to me that weight of actual dried bacterial protoplasm must be admitted to be a perfectly satisfactory measure of antigen content. It is this measure then with its correlated opacity for suspension of given weight of substance which I have taken as the basis of comparison in estimating the value of technique for giving numerical value to a suspension. The numerical values originally given by Kirwan and myself (Table I) are expressed for purposes of ready comparison in the form of a ratio to the numerical values as determined by haemocytometer.

With the method of staining and dilution above described, I found that in the case of the non-disappearing strain of *B. typhosus* the average ratio of the haemocytometer count to that in the original table was 1.75 for 20 observations. In the case of *B. paratyphosus* A the



mean of 6 observations was 1.04 and in that of *B. paratyphosus* B. 2.06 for 9 observations. And yet, by expectation, the ratios for all three organisms should have come out almost identical.

As I had observed that suspensions of certain strains of organisms showed marked discrepancies when counted by this method, it appeared probable that different results would be obtained by using other methods of staining and diluting the bacterial suspension.

This surmise was strengthened by the fact that Wright's method of counting actually gave in certain cases a consistently *higher* reading than by the hæmocytometer count when this method of staining and dilution was used.

McCoy (1917) advocates the use of a diluting fluid consisting of 0.25 c.c. of Ziehl-Neelsen's carbol-fuchsin and 100 c.c. of 5 per cent carbolic acid, freshly filtered before use. In Table II is shown the result of 126 observations upon the numerical estimation of suspensions of *B. typhosus* and *B. paratyphosus* A. and B. McCoy's staining and diluting fluid was used throughout.

The technique adopted was as follows: A suspension of a 24 hours' culture of the organism was made in normal saline solution. This suspension was standardized by means of standard opacity tubes (Brown and Kirwan 1915) and a hæmocytometer preparation was made. Eighty small squares were counted. An ordinary Thoma-Zeiss hæmocytometer was used with a well 0.1 mm. deep. All bacteria which adhered to the under-surface of the coverslip were counted separately and subsequently included in the counts.

It will be seen that the average of counts obtained when using this diluting fluid was distinctly higher than those when the previously mentioned staining and diluting procedure was adopted.

Considerable variation in the counts is also noticeable, and it might be supposed that this was largely due, either to the difficulty experienced in correctly estimating the opacity of the suspension, or to the fact that the opacity of a suspension is not a true indication of the absolute amount of bacterial substance contained.

That the variation in the counts is not due to the former cause is, I think, proved by the fact that inexperienced observers have no difficulty in arranging the opacity tubes in proper sequence, *i.e.*, they can easily discriminate one from another. That it is not due to an inconstant relationship between opacity and the amount of dried bacterial substance contained in suspension is clearly shown when we come to consider

(p. 244, § 5) the observed estimation of the opacity of a bacterial suspension and the actually weighed quantity of bacterial substance contained. It must therefore be recognised that even the method of enumeration by means of a hæmocytometer, which is now almost generally accepted as being the most accurate means of numerical estimation that we possess, is capable of giving very variable results.

### III. THE GRAVIMETRIC METHOD.

There is probably no substance used, the accurate standardization of which is more important than that of the various forms of tuberculin. This has been invariably standardized by weight.

Wilson and Dickson (1912) advocated the application of the gravimetric method to the standardization of vaccines in general. It is strange that bacteriologists still adhere to the standardization expressed in terms of number and not of weight.

It may be that in many small laboratories, where vaccines are made, no means of desiccation and no accurate balance are available. There is however at least one indirect, but perfectly satisfactory, method of judging of weight which obviates the use of such apparatus. I refer to the determination of weight of *dried* substance contained in suspension from the opacity of a suspension of moist growth.

### IV. THE RELATION OF THE OPACITY OF A SUSPENSION TO THE WEIGHT OF DRIED BACTERIAL PROTOPLASM CONTAINED.

I have already shown, Brown (1914) and Brown and Kirwan (1915), that a very close relation exists between the opacity of a bacterial suspension and the weight of dried bacteria contained. Since those papers were written I have had greater opportunity of verifying this fact, and I now propose to consider the means by which, in my opinion, a vaccine can be more accurately standardized than by any of the recognised methods of direct enumeration.

(1) *Method of standardization by means of opacity tubes.*—The method essentially consists in comparing the opacity of a bacterial suspension with that of a series of tubes containing varying dilutions of barium sulphate. The original series, described in the paper by Kirwan and myself, consisted of 9 tubes containing dilutions varying from an 8-fold to a 16-fold dilution of 1 per cent barium sulphate suspended in a 1 per cent aqueous solution of sodium citrate. This method has been extensively used, but owing to the difficulty which some observers find in

discriminating between the tubes containing the more dilute suspensions, I now advocate the use of a series of 10 tubes containing a somewhat different series of dilutions of barium sulphate suspended in 1 per cent solution of sodium citrate.

The first tube of the series contains an 8-fold suspension of 1 per cent barium sulphate and therefore corresponds exactly with the first tube of the old series, the next contains 9 volumes of this and 1 volume of citrate and so on to the last tube, which contains 1 volume of the same barium sulphate suspension and 9 volumes of citrate. Thus the second tube in comparison with the first tube contains 90 per cent barium sulphate, the third tube 80 per cent and so on down the scale.

(2) *Keeping qualities of the barium sulphate suspension.*—I have compared the opacity of freshly made suspensions of barium sulphate with others which I made 5 years ago and have found that the opacity in the two cases is identical.

(3) *Preparation of the opacity tubes.*—A strong solution of barium chloride is made and to this is added an excess of sulphuric acid. The mixture is then boiled and the precipitate is poured on to a filter paper and is washed with tap water until the filtrate is neutral to litmus paper.

The barium sulphate is then dried and thoroughly roasted. When cool a portion is accurately weighed and placed in a perfectly clean mortar. The powder is finely ground and the requisite amount of 1 per cent aqueous solution of sodium citrate is gradually added. From this 1 per cent suspension of barium sulphate, an 8-fold dilution is made and similarly the other dilutions, the sodium citrate being used throughout as the diluting fluid.

The most convenient size of tubing to be used is one of approximately 4 mm. internal diameter and about 8 cm. long. Any set of tubes prepared must be made from the same piece of glass tubing. The tubes having been filled to three-quarters of their length are sealed and numbered from 1 to 10. Several tubes from the same portion of tubing, sealed at one end, are prepared at the same time and one of these is filled at the time of standardization with the vaccine to be tested.

(4) *Technique of standardization.*—The contents of the opacity tubes having been thoroughly shaken, the two tubes to be compared are placed side by side, in a good light, upon some clearly printed book. The lighting must be equal in the two cases. The opacity of the two suspensions can then be readily compared by rolling the two tubes

from side to side, and raising them slightly from the surface of the print.

If the vaccine to be standardized has an opacity greater than that of any of the tubes, then one volume is placed in a testing tube and successive volumes of diluent added until the resulting opacity corresponds to one of the tubes of the series. The value in terms of weight of dried bacteria to be given to the test suspension is then a matter of simple calculation with the help of Table III.

It has been suggested to me that the opacity of a vaccine, prepared from a moist growth, is not necessarily the same as that of a suspension prepared by emulsifying the same quantities of bacteria in the dried state in the same volume of fluid.

If this surmise was correct then it would not be possible, by means of opacity, to standardize vaccines prepared from bacteria in the moist state.

I have frequently proved that the opacity of a suspension, made from a weighed quantity of moist growth in a given quantity of fluid, is identical with the opacity of the suspension made from that same quantity of moist growth when completely desiccated and suspended in the same quantity of fluid. In other words, the opacity of a suspension of moist growth is a measure of the quantity of dried growth contained. What does vary, under different circumstances, is the loss of weight by desiccation of a moist growth, but this fact does not in the least detract from the usefulness of opacity readings for estimation of the weight of bacterial substance in the dried form contained in suspension. As weight of bacterial substance in the dried form is logically the best measure of antigenic quantity, we have then in the opacity method of estimation an easily applied means of obtaining with accuracy the desired datum.

(5) *Constancy in the relation of the opacity of a bacterial suspension to the weight of dried bacterial protoplasm per unit volume.*—In order to test further this relationship I, first of all, made a series of 23 observations upon the opacity of 1 mgm. of dried *B. paratyphosus* B., suspended in 1 c.c. of 0·85 per cent salt solution. The technique adopted was as follows:—

The 24 hours' growth was carefully removed from the surface of a dried nutrient agar slope. This was spread on the inner surface of a dry and accurately weighed specific gravity bottle, which was allowed to remain overnight in a vacuum desiccator over sulphuric acid. The weight of the dried growth was then estimated and a suspension

made containing 1 mgm. per c.c. of 0.85 per cent salt solution. Tubes containing each of these suspensions, as made, were handed to an independent observer who pronounced them to be indistinguishable in opacity.

The relationship of opacity to the weight of dried bacterial protoplasm of the organisms, commonly used in vaccine therapy, is shown in Table III.

Although, in the case of those organisms which grow readily upon ordinary nutrient agar, no discrepancy was ever observed in the relationship between the opacity and weight per unit volume, when I came to repeat the procedure with the hæmophilic organisms, I found that certain variations did occur in this respect. That this was not due actually to the blood agar medium is, I think, shown by the fact that, when organisms of the typho-coli group were grown both on ordinary agar and upon blood agar and suspensions were made in the manner described, the opacity of the suspensions were identical and were therefore apparently independent of the medium upon which the organism had been grown. I am of opinion that these variations may possibly be due to the difficulty experienced in obtaining suspensions, in a finely divided form, of some of these organisms, more especially in the case of the gonococcus and some strains of *M. catarrhalis*.

Another factor which plays an important part in this respect is, that in the case of the hæmophilic organisms the growth is by no means luxuriant and therefore the quantities of organisms weighed and made up into suspensions have naturally to be small, thus increasing the chances of technical error.

The actual observations made showing the relationship of opacity to weight in the case of the hæmophilic organisms, is shown in the form of a frequency distribution table—Table IV.

Another point, which is shown in this table, is that the variety of *M. catarrhalis* which grows well on ordinary agar, has a greater opacity for the same weight per unit volume than that which is only capable of growing on blood agar.

(6) *Method of standardization and preparation of actual doses.*—Supposing that it is desired to standardize a staphylococcus aureus vaccine and to prepare five graduated doses. We will presume that the vaccine is equal in opacity to a No. 8 tube in the table. On referring to column K, Table III, we see that the vaccine contains 0.91 mgm. of dried staphylococcus aureus per c.c.

Supposing that the actual doses, which it is desired to prepare, are 0.03, 0.06, 0.08, 0.15 and 0.2 mgms., it will be convenient to prepare two dilutions of the vaccine, the first containing 0.1 mgm. per c.c. and the second 0.2 mgm. per c.c.

I am indebted to Lieut.-Colonel W. F. Harvey, I.M.S., for showing me a simple method by which any one dilution can be prepared from any stronger dilution.

In this case we have a suspension containing 0.94 mgm. per c.c. and require to make one containing 0.2 mgm. per c.c. In order to do this, take 0.2 c.c. of the vaccine and add to it  $0.94 - 0.2 = 0.74$  c.c. of carbolised saline.

In actual practice it would be more convenient to put 2 c.c. of the vaccine in a sterile test tube and add to it 7.4 c.c. of carbolised saline.

The dilution containing 0.1 mgm. per c.c. can be similarly prepared. Of this 0.3 c.c. would be the first dose, 0.6 c.c. would be the second, and so on.

The initial doses of influenza shown in Table III are those recommended by Ford Robertson (1918).

The figures showing the weight in milligrammes, corresponding to opacity in Table III, have been arrived at by calculation from the opacity of 1 mgm. of dried bacterial substance per c.c. of 0.85 per cent salt solution.

TABLE I.

Showing the relation of opacity to the weight and numerical equivalent of various bacteria.

(The figures underlined represent the numerical equivalent in millions of 1 mgm. of dried bacterial substance in 1 c.c. of the bacterial suspension.)

	Dilution of 1 % barium sulphate in 1 % sodium citrate.	<i>Staphylococcus</i> <i>aureus</i> .	<i>Staphylococcus</i> <i>albus</i> .	<i>B. typhosus</i> .	<i>B. coli</i> .	<i>B. paratyphosus</i> A.	<i>B. dysenteriae</i> Flexner.	<i>B. pyocyaneus</i> .	<i>B. pneumoniae</i> Friedlander.	<i>B. proteus vulgaris</i> .
18	..	.. 3,374	3,150	2,138	1,951	2,044	2,633	5,250	3,120	2,479
19	..	.. <u>3,000</u>	2,800	1,900	1,734	1,817	2,341	4,666	2,773	2,204
110	..	.. <u>2,500</u>	2,520	1,710	1,560	1,635	2,107	4,200	2,496	1,983
111	..	.. 2,454	2,290	1,555	1,419	1,487	1,915	3,818	2,269	1,803
112	..	.. 2,250	2,100	1,425	1,300	1,363	1,739	3,500	2,180	1,653
113	..	.. 2,076	1,938	1,315	1,200	1,258	1,621	3,230	1,920	1,526
114	..	.. 1,928	1,800	1,221	1,115	1,169	1,442	3,000	1,783	1,417
115	..	.. 1,800	1,680	1,140	1,040	1,090	1,405	2,800	1,664	1,322
116	..	.. 1,687	1,575	1,069	975	1,022	1,317	2,625	1,560	1,240



TABLE II.

*Showing frequency distributions of ratios of counts obtained by a hæmacytometer method to original counts obtained by the different method of Table I.*

Ratio of the numerical content of bacterial suspensions by hæmacytometer method to original value (Table I) taken as unity.	B. typhosus.	B. paratyphosus A.	B. paratyphosus B.
2.9	..	..	..
2.8	1	..	..
2.7	..	..	..
2.6	..	1	..
2.5	3	2	3
2.4	6	5	2
2.3	4	9	3
2.2	8	11	3
2.1	4	7	11
2.0	4	6	5
1.9	3	6	5
1.8	2	1	1
1.7	4	2	2
1.6	..	..	1
1.5	..	..	..
1.4	..	..	1
1.3	..	..	..
MEAN ..	2.12	2.15	2.07



TABLE III.

Showing the relation of opacity to the weight of dried bacterial substance expressed in milligrammes per cubic centimetre of bacterial suspension.

Per-centage of Ba SO <sub>4</sub> suspension.*	A	B	C	D	E	F	G	H	I	J	K	L	Organism.	Column.
100 "	2.40	2.22	2.04	1.96	1.72	1.46	1.56	1.43	1.38	1.33	1.18	1.05	M. catarrhalis on blood agar	A
90 "	2.14	2.00	1.84	1.76	1.55	1.50	1.41	1.29	1.24	1.20	1.06	0.95	S. cholerae	B
80 "	1.90	1.80	1.63	1.57	1.38	1.33	1.25	1.14	1.10	1.07	0.94	0.84	B. influenzae	C
70 "	1.70	1.55	1.43	1.37	1.20	1.16	1.09	1.00	0.96	0.93	0.82	0.74	Pneumococcus	D
60 "	1.43	1.33	1.22	1.18	1.03	1.00	0.94	0.86	0.83	0.80	0.71	0.63	B. pyocyaneus & meningococcus	E
50 "	1.20	1.11	1.02	1.00	0.86	0.83	0.78	0.71	0.69	0.67	0.59	0.53	Streptococcus pyogenes	F
40 "	0.95	0.90	0.80	0.79	0.69	0.66	0.62	0.57	0.55	0.53	0.47	0.42	M. catarrhalis (on ordinary agar)	G
30 "	0.71	0.67	0.60	0.59	0.52	0.50	0.47	0.43	0.41	0.40	0.35	0.32	B. dysenteriae Shiga & Flexner.	H
20 "	0.48	0.44	0.40	0.39	0.34	0.33	0.31	0.29	0.28	0.27	0.23	0.21	B. typhosus, B. paratyphosus	I
10 "	0.24	0.22	0.20	0.19	0.17	0.16	0.16	0.14	0.14	0.13	0.12	0.10	B. paratyphosus B. and B. coli	J
													M. melitensis	K
													Staphylococcus aureus	L
													Staphylococcus albus	

Initial dosage recommended expressed in milligrammes.

#### Prophylactic.

B. typhosus	0.15													
B. paratyphosus A	0.1													
B. paratyphosus B	0.1													
S. cholerae	1													
Meningococcus	0.08													
B. influenzae	0.03													
Pneumococcus	0.06													

#### Curative.

Staphylococcus	0.03													
Streptococcus pyogenes	0.003													
B. coli	0.01													
B. pyocyaneus	0.003													
Gonococcus	0.002													
Pneumococcus	0.017													
B. influenzae	0.005-0.01													
M. melitensis	0.002													
B. typhosus	0.015													
M. catarrhalis	0.01													

\* The Ba SO<sub>4</sub> suspension consists of an 8-fold dilution of a 1% suspension of Ba SO<sub>4</sub> in 1% sodium citrate solution.

TABLE IV.

*Showing the frequency distribution of variations in opacity of suspensions of different hæmophilic organisms. The suspensions throughout were made up at 1 mgm. of dried bacterial substance per c.c. of diluent. Intermediate values between actual tubes were evaluated by interpolation.*

Opacity tube number.	Pneumo-coccus.	Strepto-coccus pyogenes.	Gonococcus.	Influenza.	M. catarhalis (blood agar).	M. catarhalis (ordinary agar).
7.25	..	..	..	..	..	..
7	1	..	..	..	..	..
6.75	..	..	..	..	..	..
6.5	2	5	2	..	..	5
6.25	..	..	..	..	..	..
6	2	..	..	..	..	1
5.75	..	..	..	..	..	..
5.5	3	3	..	..	..	..
5.25	..	..	4	3	..	..
5	1	..	..	5	1	..
4.75	..	..	..	..	..	..
4.5	1	..	2	3	7	..
4.25	..	..	..	..	..	..
4	..	..	2	..	4	..
3.75	..	..	..	..	..	..
3.5	..	..	..	..	2	..
3.25	..	..	..	..	..	..
3	..	..	..	..	..	..
MEAN ..	5.8	6.1	5.1	4.9	4.2	6.4

## CONCLUSIONS.

1. The results of standardization of bacterial suspensions, in terms of their numerical equivalents, vary considerably and are dependent upon the method used.

2. When using the hæmocytometer method the nature of the staining and diluting fluids produces a considerable effect on the numerical count.

3. The method of staining a suspension by mixing it with an equal volume of freshly filtered carbol-fuchsin, and making the subsequent dilutions in distilled water, is an unsatisfactory procedure in that very irregular results may be obtained by this method.

4. The figures given in Table I were essentially arrived at from results of standardization by Wright's method, and, although these figures are much lower than those which would have been obtained when using the hæmocytometer method, it must be remembered that the majority

of the doses of vaccines now recommended were based on the results of standardization by Wright's method.

5. The opacity of a bacterial suspension is intimately correlated with the weight of *dried* bacterial protoplasm contained.

6. The opacity of the barium sulphate suspension is permanent, a freshly made suspension being indistinguishable from one five years old.

7. Standardization by means of the estimation of the opacity of a bacterial suspension is a rapid and accurate method of determining the weight of *dried* bacterial protoplasm contained in the suspension.

I wish to express my gratitude to Lieut.-Colonel W. F. Harvey, I.M.S., Director, Central Research Institute, Kasauli, for his valuable help and criticism during the preparation of this paper.

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# ON THE POSSIBLE SPREAD OF SCHISTOSOMIASIS IN INDIA.

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THERE are few problems in medical zoology as yet opening before us of which the purely zoological aspect is more complex than that of the etiology of the various forms of Schistosomiasis or Bilharziasis. To reach the most elementary basis for any final solution we must have in the first instance sure and detailed information as to several very difficult points in taxonomy. Not only must we be able to distinguish the parasites of the disease in their different stages and generations, but we must be able to identify with certainty their intermediate snail hosts.

In a paper recently published by one of the most illuminating of the American writers on Trematodes,<sup>1</sup> stress is laid on the necessity of careful descriptions of the parasites. "A mere superficial description of the worm is a distinct burden on the literature. The cercaria should be carefully studied in minute detail or not at all.... It is necessary, then, to urge the investigator in this group to use the utmost care in his

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<sup>1</sup> Faust, *American Naturalist*, LIII, p. 89 (1919).

work, to describe the minute parts of the organs, and to record the complete biological data available that these records may be of use in life-history investigations." Equal importance must be attached to the identification and description of the intermediate host; and this is a much less simple matter than it appeared until recently to be, for we now have full proof that it is useless to attempt to classify, and in many cases even to identify, the freshwater molluscs without considering the soft parts of the animal as well as the structure of the shell. If we can be sure that a given parasite cannot reach the stage at which it becomes dangerous to man except through a given snail, the presence or absence of that snail in a given locality is a matter of the first practical importance.

When, therefore, in May 1918, the Zoological Survey of India—a department, be it remembered, consisting at present of only four scientific officers—was invited to assist the medical authorities in discovering all that could be discovered about the introduction from abroad and possible spread of *Schistosomiasis* in India, the first point considered was the distribution and mode of occurrence in the country of the freshwater molluscs, for this was a subject on which a considerable amount of information was already available and more was in the course of acquisition. A survey of the freshwater molluscs of India was immediately decided upon, and is now making good progress with the assistance of naturalists both in India and Europe. At the same time the recent work of American parasitologists greatly facilitated an enquiry into the distribution and specific characters of the Indian trematode cercariae, and the infection experiments of Leiper did not appear impossible of imitation, provided that a patient suffering from the disease could be obtained.

The main object of this paper is to put on record our infection experiments, which though negative in their results are not altogether devoid of interest. Before coming to them, however, it may be as well to give a brief résumé from a zoological point of view of the whole problem, which can only be appreciated at present by a study of much scattered literature, to some of which it is difficult to obtain access.

It must be borne in mind that a large number of the statements published on the zoological aspect of *Schistosomiasis* are found on analysis to be based on observations never followed out in detail, or to depend on assumptions which are altogether unproven. Many of them, notwithstanding, have obtained a large measure of acceptance and

pass as current knowledge. In considering what has been written on the subject it is most necessary to discriminate between grain and chaff.

It is only within the last few years that it has been definitely proved that Schistosome worms, like other digenetic trematodes, pass a portion of their life-cycle in freshwater molluscs. Miyairi and Suzuki, writing in Japanese in 1913, were the first to establish this fact and to follow out the full development of any species of Schistosome. Leiper's subsequent researches in Egypt and in the Far East have thrown much additional light on the subject.

The general course of development appears to be the same in all Schistosomes. The eggs pass out from the definitive host with the urine or faeces and, on falling into water, hatch in a very short time. The young larvæ or miracidia liberated in this way swim actively by means of the cilia which clothe their bodies. On coming in contact with a suitable species of freshwater snail the miracidia bore their way into it and undergo further development in the digestive gland. The miracidia develop into sporocysts, simple elongate sacs which are distinguished from rediæ (the corresponding stage in many other trematodes) by the complete absence of the pharynx and gut. The sporocyst by internal budding gives rise to daughter sporocysts and each of these by a similar process produces a number of cercariæ. The cercaria of Schistosomatids is elongate and consists of two parts, a body which bears two suckers, and a long tail which is forked at the extremity. Owing to the latter peculiarity these cercariæ are termed furcocercous, but only those which are without pigmented eye-spots and are devoid of a pharyngeal bulb are considered to belong to the family Schistosomatidæ.

When the cercariæ are fully developed within the sporocyst, they escape through a rent in its wall and thence, through the rupture of the digestive gland of the snail, they are set free in the surrounding water: often they are expelled through the pulmonary aperture in a series of puffs. The cercaria, once it has left the snail, has a very short life. It swims vigorously by means of its tail and, if it meets with the definitive mammalian host, it bores its way through the skin or, when the water has been used for drinking, obtains entry by penetration of the mucous membrane of the mouth or œsophagus. In this process the tail drops off. The cercaria finds its way by the veins or through the lymph ducts to the portal system and completes its metamorphosis. The

adults are for the most part found in the liver, and in the portal and mesenteric veins and their branches; they are frequently found paired, the more slender female lying in the groove or gynæcophoric canal of the male.

Apart from its occurrence in individuals who have travelled in countries where the disease is endemic, human Schistosomiasis is practically unknown in India. Though a few cases have been recorded in persons who have never left the country, no instance is known of a localised outbreak of the disease such as would be expected if miracidia from an infected individual succeeded in establishing themselves in a suitable molluscan host. India's apparent freedom from Schistosomiasis is astonishing when it is remembered that the disease is endemic and occurs with great severity in countries both to the west and in the far east; in Egypt and other parts of Africa and in Mesopotamia on the one hand and in China and Japan on the other.

The question whether human Schistosomiasis can be transmitted in India has recently assumed great importance owing to the fact that numbers of men engaged in military operations in Egypt contracted the disease in that country and have been invalided to India. If, therefore, a suitable molluscan host exists, there is grave danger that the parasite will spread rapidly with disastrous effects on the health of the population. The habits of the lower classes in India are precisely those that would tend to the rapid dissemination of the disease were it once to gain a foothold.

At the present time the following eight species of *Schistosoma*<sup>2</sup> are known<sup>3</sup> :—

<i>S. hæmatobium</i> (Bilharz).	<i>S. bomfordi</i> (Montgomery).
<i>S. mansoni</i> (Sambon).	<i>S. indicum</i> (Montgomery).
<i>S. japonicum</i> (Katsurada).	<i>S. spindalis</i> (Montgomery).
<i>S. bovis</i> (Sonsino).	<i>S. turkestanicum</i> (Skrjabin).

<sup>1</sup> Milton, *Ind. Med. Gaz.*, XLIX, p. 10 (1914).

<sup>2</sup> For the affinities of the family Schistosomatidae and for diagnoses of the component genera, v. Odhner, *Zool. Anzeiger*, XLI, p. 54 (1913), and Johnston, *Journ. Proc. Roy. Soc. N. S. Wales*, L., p. 238 (1917). Both these authors use the name *Bilharzia* in place of *Schistosoma*, but Weinland's account of the latter appears to have been published in 1858, antedating Cobbold's description of *Bilharzia* by one year.

<sup>3</sup> Milton (*Ind. Med. Gaz.*, LIV, p. 127, 1919) mentions a *Schistosoma* with the name *S. reflexum*, 'described by certain German authors as occurring in cattle.' I have not been able to find any reference to this species. Stiles and Hassall in their *Index-catalogue of Medical and Veterinary Zoology* remark that *Schistosoma reflexum* is a term in teratology and that it has no status in nomenclature.



*S. capensis* (Harley) and *S. magnum* (Cobbold) are thought to be synonyms of *S. hæmatobium*. *S. crassa* (Sonsino) is a synonym of *S. bovis* and Blanchard's *S. cattoi* is the same as *S. japonicum*.

Only the first three of the species listed above are normally parasitic in man.

*S. hæmatobium*<sup>1</sup> has its headquarters in Egypt and is also recorded from other parts of Africa, from Mauritius, Madagascar and Mesopotamia. Leiper<sup>2</sup> has found that the intermediate hosts in Egypt are *Bullinus contortus*, *B. dybowskii* and *B. innesi*; but the two last mentioned are perhaps merely local races or varietal forms of *B. contortus* and not distinct species.<sup>3</sup> The records of *S. hæmatobium* from other parts of Africa and from Mesopotamia are based for the most part solely on the character of the egg, which is oval and possesses a single terminal spine. But this type of egg is also found in two non-human species of *Schistosoma*, *S. bomfordi* and *S. indicum*. It is therefore very desirable that the anatomy of adult worms should be examined and compared in detail with that of *S. hæmatobium*. Becker<sup>4</sup> appears to have proved that the human Schistosome in the Transvaal passes its early stages in the snail *Physopsis africana*. There is, however, nothing to show that the adult worms which he reared in a guinea-pig have been systematically examined, and it is therefore not altogether certain that he is right in referring the species to *S. hæmatobium*. Cawston<sup>5</sup> has recorded the cercaria of *S. hæmatobium* from the same species of mollusc in Natal. This statement can hardly be accepted in the present state of our knowledge, for the cercaria of *S. hæmatobium* has never been described in detail, and, apart from breeding, the only method of identification at present available—that of measurements—is of little value with such highly contractile organisms. Sturrock<sup>6</sup> has reported that a form of Schistosomiasis, caused he believes by *S. hæmatobium*, occurs in Mesopotamia and Boulenger<sup>7</sup>

<sup>1</sup> A detailed account of the anatomy of this species is given by Looss, *Arch. Mikrosk. Anat. Entwicklungs-gesch.*, XLVI, p. 1 (1895).

<sup>2</sup> Report on the Results of the Bilharzia Mission in Egypt, 1915, *Journ. R.A.M.C.*, XXV, pp. 1-55, pp. 147-192, XXVI, pp. 253-267, XXVII, pp. 171-190 (1915-16).

<sup>3</sup> See Annandale's figures illustrating variation in *B. contortus* from Mesopotamia, *Rec. Ind. Mus.*, XV, pl. XX, figs. 6-11 (1918).

<sup>4</sup> Becker, *Med. Journ. S. Africa*, XI, p. 156 and XII, p. 42 (1916).

<sup>5</sup> Cawston, *Parasitology*, XI, p. 83 (1918) and *Journ. Trop. Med. Hyg.*, XXI, p. 241 (1918).

<sup>6</sup> Sturrock, *Brit. Med. Journ.*, 1899, p. 1543.

<sup>7</sup> Boulenger, Report on Bilharziasis in Mesopotamia. *Indian Journal of Medical Research*, current number, p. 8. We have to thank Captain Boulenger for enabling us to see this.

has found that a urinary type of the disease is widely spread among the native population. The molluscan host in this country is not certainly known, for although *Bullinus contortus* occurs, having indeed been recorded from the banks of the Euphrates by Mousson in 1874,<sup>1</sup> all the specimens hitherto found have been dead and most of them in sub-fossil condition. That Captain Boulenger's extended investigations failed to result in the discovery of a single living example of *B. contortus* would tend to indicate that the mollusc, owing perhaps to some abnormal climatic conditions, has recently become very scarce in Mesopotamia. If this be so, the parasite must have adapted itself to some new molluscan host, for an outbreak of Schistosomiasis of undetermined origin occurred among the personnel of an Indian General Hospital at Basra in November 1917. Captain Boulenger notes that the only abundant molluscs in water adjacent to the hospital where the outbreak took place were species of *Neritina* and *Melanopsis*.<sup>2</sup>

Scott<sup>3</sup> has recorded a case of Schistosomiasis in a Persian officer who had never left his country; but in Eastern Persia the disease appears to be unknown. During a tour in Seistan, recently made by officers of the Zoological Survey of India, no information as to the existence of Schistosomiasis could be obtained and Major D. Heron, C.I.E., I.M.S., told us that no case had come under his notice during six years' residence in the country.

*Schistosoma mansoni* has only recently been clearly distinguished from *S. hæmatobium*.<sup>4</sup> It is found in Egypt, though less abundantly than *S. hæmatobium* and appears to be rather widely distributed in other parts of Africa. In Egypt Leiper has found that the intermediate host is *Planorbis boissyi*. *S. mansoni* is also stated to occur in the West Indies and in S. America. Manson<sup>5</sup> recorded the disease from Antigua in 1902 and the first case contracted with certainty in Guadeloupe was described by Mathis and Beaujean in 1910.<sup>6</sup> These statements, if correct, tend to the conclusion that the disease has spread from the old world to the new and that in the latter *S. mansoni* must have adapted itself to a

<sup>1</sup> Mousson, *Journ. Conchyliol.*, XXII, p. 42 (1874), under the name *Physa* (*Isidora*) *brocchii* var. *approximans*.

<sup>2</sup> They have been determined by Dr. Annandale as *Neritina jordani* and *Melanopsis nodosa*.

<sup>3</sup> Scott, *Brit. Med. Journ.*, 1904, I, p. 725.

<sup>4</sup> Leiper, *Brit. Med. Journ.*, 1916, I, p. 411.

<sup>5</sup> Manson, *Journ. Trop. Med. Hyg.*, V, p. 384 (1902).

<sup>6</sup> Mathis and Beaujean, *Bull. Soc. Med. Chirurg. Indochine*, I, p. 174 (1910).

new intermediate host. The facts are therefore of peculiar interest in India at the present time. Unfortunately the literature we have been able to consult does not afford any absolute certainty that the true *S. mansoni* is responsible for Schistosomiasis in America. The diagnosis in practically every instance is based on the structure of the egg, which bears a lateral spine, and, as with *S. haematobium*, it may be doubted whether a single character in the egg is sufficient for precise identification. It appears to us most necessary that adult worms from America should be compared in detail with authentic specimens of *S. mansoni* from Africa. Iturbe and Gonzalez, in a paper we have not been able to consult,<sup>1</sup> have recorded the early stages of *S. mansoni* in Venezuela in *Planorbis guadelupensis*. Some of these authors' material has, however, since been examined by Faust<sup>2</sup> who finds that it contains a totally different parasite belonging to the genus *Tetracotyle*. Faust notes that this *Tetracotyle* is figured by Iturbe and Gonzalez as the 'redia' of *S. mansoni*. The work in Venezuela is not therefore very convincing. Lutz<sup>3</sup>, in Brazil, appears to have succeeded in rearing the parasite which causes Schistosomiasis in S. America. Snails, identified as *Planorbis olivaceus*, were artificially infected with miracidia and with the cercariae that developed rabbits, guinea-pigs and other animals were inoculated. In from five to six weeks numerous adult worms were found in the mammalian hosts. *Planorbis olivaceus*, as Leiper has remarked, is very similar to, if not identical with, *P. guadelupensis*.

*Schistosoma japonicum* was described by Katsurada in 1904.<sup>4</sup> The disease caused by it occurs with great severity in some parts of Japan and is common in China, particularly in the Yang-tse Valley. It has also been recorded from the Philippine Islands. Miyairi and Suzuki<sup>5</sup> in 1913 succeeded in tracing the development of the parasite through a small Hydrobiid snail 'with dark-coloured shell and seven spirals.' They inoculated mice with cercariae derived from the snail and reared adult worms. It is generally agreed that the species which causes Schistosomiasis in China is the same as that found in Japan. *S. cattoi*, Blanchard,

<sup>1</sup> Iturbe and Gonzalez, *Nat. Acad. Med. Caracas*, 1917.

<sup>2</sup> Faust, *Journ. Parasitology*, IV, p. 109 (1918).

<sup>3</sup> Lutz, *Brazil Medico*, XXX, p. 385 and XXXI, pp. 81, 89 (1916-17), see abstract in *Trop. Dis. Bull.*, IX, p. 271 and XI, p. 78 (1917-18).

<sup>4</sup> Katsurada, *Annot. Zool. Japon.*, V, p. 147 (1904).

<sup>5</sup> Miyairi and Suzuki, *Tokio Med. Journ.*, 1913 (in Japanese), see abstract in *Trop. Dis. Bull.*, III, p. 289 (1914), also *Mitt. Med. Fak. Univ. Kyushu Fukuoka*, I, p. 187 (1914), abstracted in *Trop. Dis. Bull.*, VIII, p. 506 (1916).

the name given to the parasite in the former country, is regarded as a synonym of *S. japonicum*. Leiper and Atkinson,<sup>1</sup> using eggs derived from a dog infected in China, were successful in rearing cercariæ in a Japanese snail described by Robson as *Katayama nosophora*. This mollusc, which is sometimes erroneously called *Blanfordia nosophora*, is probably the same as that employed by Miyairi and Suzuki.<sup>2</sup> According to Annandale it should be referred to the genus *Hypsobia*.<sup>3</sup> The intermediate host in China appears to be unknown, but two other species of *Hypsobia* are known from the Yang-tse Valley.<sup>4</sup>

It will be noted that the molluscs which have been recorded as intermediate hosts for the species of *Schistosoma* parasitic in man are by no means closely related zoologically. *Hypsobia* belongs to the family Hydrobiidæ and to the order Pectinibranchiata, while *Bullinus* and *Planorbis* belong to the family Planorbidæ and the order Pulmonata. *Physopsis* has been referred to the family Physidæ of the latter order, but its precise position must remain uncertain until the anatomy has been worked out.

From the foregoing summary it will be observed that, even if the human parasites alone are considered, there is little ground for the theory that the molluscan host is specific. The records, it is true, are frequently open to question and much further work is necessary before many of the published statements can be accepted without demur; but it will be seen that both for *S. hæmatobium* and *S. mansoni* more than one intermediate host has been cited and it is clear that Schistosomiasis in China is carried by some mollusc other than the exclusively Japanese *Hypsobia nosophora*. Our knowledge of the general course of development in digenetic trematodes does not support the view that the intermediate host is necessarily specific. It is by no means infrequent to find cercariæ of the same trematodes in two or more species of mollusc: several instances have come to our notice in examining Indian freshwater snails. Cort,<sup>5</sup>

<sup>1</sup> Leiper and Atkinson, *Brit. Med. Journ.*, 1915, 1, p. 201.

<sup>2</sup> The species was described almost simultaneously by Robson (*Brit. Med. Journ.*, No. 2822, p. 203) as *Katayama nosophora* and by Pilsbry (*Nautilus*, XXIX, p. 1) as *Blanfordia nosophora*. The two descriptions seem to have been issued quite independently and the two authors to have selected the same specific name fortuitously. Pilsbry's description was published in May 1915 and Robson's in January of the same year. The latter description has, therefore, priority and the species must be known as *Hypsobia nosophora* (Robson). N. A.

<sup>3</sup> Annandale, *Mem. Asiat. Soc., Bengal*, VI, p. 306 (1918).

<sup>4</sup> *H. lemnida*, Heude and *H. minuscula*, Annandale (*v. Annandale, loc. cit.*).

<sup>5</sup> Cort, *Journ. Parasitology*, IV, p. 172 (1918).

moreover, in an interesting note on the adaptability of *Schistosoma* larvæ to new hosts, mentions that three American (non-human) *Schistosomatids* are known to occur in more than one species of mollusc, one indeed having been discovered in *Planorbis*, *Limnaea* and *Physa*, genera belonging to three distinct families. All that is really certain about this aspect of the case is, therefore, that very few freshwater molluscs are adapted to act as intermediate hosts for the various forms of *Schistosoma* and that the species so adapted are not the same for different species of parasite.

None of the species of mollusc which have been cited as intermediate hosts for the human forms of Schistosomiasis have been found in India and that any of them will henceforth be discovered is improbable. The genus *Planorbis* (*sensu lato*) has numerous representatives in India; but *Bullinus*,<sup>1</sup> *Physopsis* and *Hypsobia* are not known to occur. Notwithstanding these facts there is from a zoological point of view no reason to discredit the idea that a potential intermediate host may exist and that the return of infected troops from Egypt will possibly result in an outbreak of Schistosomiasis in this country.

It has now been conclusively shown that trematode cercariæ exhibit marked differences in structure and that it is possible even in this stage to distinguish the species with certainty. For the very considerable advances that have recently been made in this direction much credit is due to American parasitologists, and particularly to Cort and Faust.<sup>2</sup> At the present moment the situation is anomalous to a degree. For although we possess detailed information regarding the structure of a large number of cercariæ that have no connection with human disease—information that will certainly enable them to be recognised without difficulty on future occasions—the anatomical peculiarities of the cercariæ of *Schistosoma hæmatobium* and *S. mansoni* are practically unknown. The cercaria of *S. japonicum* has recently been described with a wealth of anatomical detail by Cort<sup>3</sup> and were this parasite to

<sup>1</sup> This genus seems to be one of discontinuous distribution. It occurs in Africa and Mesopotamia and, though not known with certainty further east on the continent of Asia, reappears with an abundance of species in Australia. A gigantic fossil shell probably belonging to the genus is, however, common in the Eocene beds of Nagpur, Baluchistan, etc.

<sup>2</sup> The principal papers are, Cort, *Illinois Biol. Monogr.*, I, p. 447 (1915); *Journ. Parasitology*, I, p. 65 (1914), *ibid.*, IV, p. 49 (1917), *ibid.*, V, p. 86 (1918); Faust, *Illinois Biol. Monogr.*, IV, p. 1 (1918); *Journ. Parasitology*, III, p. 105 (1917), *ibid.*, IV, pp. 94 and 148 (1918).

<sup>3</sup> Cort, *Univ. California Public. Zool.*, X, <sup>11</sup>11, p. 485 (1919).



establish itself in India we should have no difficulty in its identification. But we have no similarly detailed knowledge of the cercariæ of *S. hæmatobium* and *S. mansoni*<sup>1</sup> and in practice the only way by which we can attempt to distinguish them from one another or from other species of the same family is by a system of measurements, a method which is very unreliable with such contractile organisms.

The experiments which we have carried out in the attempt to determine whether a potential molluscan host for Schistosomiasis exists in India were commenced at the end of August 1918. We were informed by the medical authorities that at Hyderabad (Deccan) there were many men who had been enlisted by H. E. H. the Nizam, had served in Egypt, and had been invalided to India suffering from the urinary type of the disease. Towards the end of August one of us visited Hyderabad on tour, accompanied by Dr. Annandale, who was at the time engaged on a survey of the molluscs of S. India. Through the kindness of Dr. A. Lankester, Director of the Medical and Sanitation Department, Hyderabad State, we obtained the services of a man who had contracted the disease near Suez. Dr. Lankester informed us that over one hundred men suffering from Schistosomiasis were then living in the vicinity of Hyderabad.

Freshwater molluscs were collected in numerous localities in the neighbourhood of Hyderabad and Secunderabad and the results of the enquiry, so far as the molluscs themselves are concerned, will in due course be published by Dr. Annandale. The observations made lead us to believe that during 1918 there was less danger than might ordinarily be expected of an outbreak of Schistosomiasis. Owing to the exceptional drought the ponds and other bodies of water were in most places greatly reduced in size even if not altogether dry and the vast numbers of dead shells round their margins showed that the mortality among the molluscan fauna had been unusually heavy. In some of the ponds practically every mollusc appeared to have perished. It is of course evident that any diminution in the danger caused by drought is purely temporary in character, for it may be expected that molluscs

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<sup>1</sup> Col. P. Bahr, R.A.M.C., has kindly sent us extracts from a paper shortly to be published by him and Major N. A. Fairly, A.A.M.C., together with photographic copies of the plates that will accompany it. This paper, among much other valuable matter, contains a far more detailed account of the anatomy of the cercariæ of *S. hæmatobium* and *S. mansoni* than any that has hitherto appeared.

will multiply and become as abundant as ever, as soon as conditions become favourable.

Thanks to facilities provided by Captain A. J. Powell and other R.A.M.C. officers attached to the Hislop War Hospital, it was possible to investigate the trematode larvæ in the freshwater snails of the localities, and attempts were also made to infect several species with *Schistosoma* miracidia obtained from the patient supplied by Dr. Lankester. The latter experiments were for the most part robbed of any possibility of success owing to the fact that the great majority of the snails died during transit to Calcutta. This appeared to be due, not so much to overcrowding, as to the harmful effects of the vibration to which they were subjected in the course of a long railway journey. The patient suffering from *Schistosomiasis* was, however, brought to Calcutta, and further infection experiments were made on a number of local species of mollusc in the laboratories of the Zoological Survey of India.

In conducting the experiments it was necessary in the first place to discover what trematode parasites normally occur in the snails. Numbers of specimens of a particular species were therefore dissected and examined microscopically and the extent of infection with one or more species of trematode determined. At the same time attempts were made to infect examples of the same snail from the same locality with *Schistosoma* miracidia. The urine of our patient, which contained large numbers of terminal-spined eggs, was centrifuged and the sediment placed in a watch-glass with a little water. The eggs were watched under a low power and in the course of a period varying from fifteen minutes to half an hour the water swarmed with miracidia. A number of molluscs (usually about thirty in the case of the smaller species) were then placed in a vessel with the smallest possible quantity of water and the miracidia were added. Leiper found that, in those of his experiments which were successful, the miracidia were strongly attracted by the mollusc; but though careful watch was kept no such attraction could be detected with any of the species which we employed.

After several hours in contact with the miracidia the snails were transferred to aquaria. Some of the smaller forms were kept in large glass jars, but the larger species were put into earthenware pots  $2\frac{1}{4}$  feet in diameter and  $1\frac{1}{2}$  feet high. All the aquaria were supplied with selected water-weeds and with covers of mosquito netting. They were placed out of doors in a situation where sun reached them only for a short



time in the early morning. After a period varying from four to eight weeks the molluscs were dissected and carefully examined for parasites.

On p. 264a will be found a table indicating the varieties of trematode larvæ found in the species of mollusc<sup>1</sup> that formed the subject of our infection experiments and the number of parasitized specimens obtained in each locality. The trematode larvæ are classified according to the scheme proposed by Lühe.<sup>2</sup> It will be noticed that the degree of infection varies very greatly with different molluscs. Sometimes, as with *Vivipara bengalensis* and certain species of *Limnæa*, trematode parasites appear to be altogether absent; sometimes, as with *Melania lineata* and *M. tuberculata* from Waltair, 85 to 90 per cent are infected. It is hoped, at a later date, to publish a detailed systematic account of these larval trematodes and of others obtained during the course of the mollusc survey.

Schistosome larvæ belong to the Distome group and, as already explained, are termed 'furcocercous.' It will be seen from the table that, among the species of mollusc employed in our experiments, furcocercous larvæ were found to occur as a natural infection only in three. They were observed in *Melania tuberculata* from Trimulgherry (once only in a mixed infection), in *Planorbis exustus* from Bolarum (twice only) and in the same species from Calcutta.

The furcocercous cercariæ obtained from Calcutta specimens of *Planorbis exustus* resemble those of the family Schistosomatidæ, but in size and proportionate measurements they do not agree with the descriptions of the cercariæ of any of the human parasites. They are much larger and the flukes are almost as long as the undivided portion of the tail. These cercariæ were abundant in Calcutta last August and 23 per cent of *Planorbis exustus* in the tank in the compound of the Indian Museum were found to be infected. We thought it probable that this cercaria would prove to be identical with that which Liston and Soparkar<sup>3</sup> state to belong to *Schistosoma spindalis*, for this larva

<sup>1</sup> In the preparation of this paper we have throughout received much valuable assistance from Dr. Annandale. He has always been ready to place his special knowledge of the mollusca at our disposal and we are indebted to him for the identification of the species listed in this table.

<sup>2</sup> Lühe, *Parasitische Plattwürmer*, I, Trematodes, in *Die Süßwasser fauna Deutschlands* summarised by Cort, *Illinois Biol. Monogr.*, I, p. 64 (1915).

<sup>3</sup> Liston and Soparkar, *Ind. Journ. Med. Research*, V, p. 567 (1918).

was also obtained in *Planorbis exustus*. Dr. Soparkar has, however, kindly sent us specimens of the cercariæ of *S. spindalis* and we find on comparison that they differ conspicuously from our Calcutta material.

Of the two *P. exustus* from Bolarum infected with furcocercous larvæ, one was found to contain the same species as that found in this mollusc in Calcutta. The other specimen and the *Melania tuberculata* from Trimulgherry were infected with very peculiar furcocercous forms which evidently do not belong to the genus *Schistosoma* and perhaps not even to the family Schistosomatidæ.

It will thus be seen that of all the trematode larvæ found to exist as natural infections in the molluscs we experimented with, none can be suspected of any connection with human Schistosomiasis.

The mere fact that we failed to find the cercariæ of any human Schistosome in the neighbourhood of Secunderabad, where numerous infected persons were living, is of course altogether inconclusive. We do not pretend to have examined all the bodies of water in the neighbourhood, the scarcer forms of mollusc were frequently not obtained in sufficient numbers for experimental purposes and the incidence of trematode infection in the mollusc is probably seasonal and might for this reason have escaped detection.

The details of our attempts to infect molluscs artificially with miracidia of human Schistosomiasis are shown in the table on p. 264*b*. The methods we employed have already been explained.

Our results are entirely negative. We obtained no evidence that the urinary form of the disease is capable of transmission by any of the molluscs we used. Only in one instance was a furcocercous cercaria found, occurring as a mixed infection with a small monostome in a single specimen of *Melania lineata*. We are satisfied that this cercaria is not that of a human Schistosome and it is indeed evident, from the very heavy artificial infection to which the molluscs were subjected, that if the species were capable of transmitting the parasite a much heavier percentage of infected snails would have been found.

It must not be thought that our results indicate that the species used in our experiments are necessarily incapable of acting as intermediate hosts. In a number of instances the mortality among the molluscs was so high that the results cannot be regarded as satisfactory. Leiper notes, moreover, that the cercariæ which cause human Schistosomiasis

in Egypt were not to be found in the snails at all times of the year, and it is probably true that trematode larvæ in general are seasonal in their appearance. Our experiments, therefore, need repetition, especially at different times of the year, before it can be assumed that any of the molluscs we employed are harmless as transmitters of disease.

TABLE II.

Showing the larval trematodes found in molluscs after attempts at infection with *Schistosoma miracidia*.

Species.	Locality.	Date of infection with <i>Schistosoma miracidia</i> .	Number infected.	Date of examination.	Number surviving at date of examination.	Results of examination.
		1918		1918		
<i>Melania tuberculata</i> (Müller)	Hialop War Hospital, Trimulgherry, Secunderabad.	3-ix	16	30-x	7	One infected with large Monostome, remainder without trematode parasites.
Do.	Basin in fern-house, Indian Museum, Calcutta. [Small specimens.]	6-x	50	7-xii	40	Three infected with large Monostome; remainder without trematode parasites.
Do.	Do. [Medium and large specimens.]	4-x	20	9, 32-xii	13	Nine infected with large Monostome; remainder without trematode parasites.
<i>Melania variabilis</i> , Benson	Tank, Indian Museum, Calcutta.	24-ix	30	{ 23-xi, 2-xii	20	Six infected with Cercariae; remainder without trematode parasites.
<i>Melania lineata</i> , Gray	Do.	25-ix	30	6-xii	23	Seventeen infected with small Monostome, two with a megalurous Distome and one with a mixed infection of the small Monostome and a furcocercous Distome. The three remaining specimens without trematode parasites.
<i>Amnicola orcula</i> , Benson	Pond near railway station, Waltair.	14-ix	20	1-xi	9	One infected with a small number of rediae and a few unhealthy gymnocephalous cercariae with eye-spots. The remainder without trematode parasites.
Do.	Do.	18-ix	12	21-xi	4	No trematode parasites.
Do.	Tank, Indian Museum, Calcutta	21-ix	30	20-x	14	One infected with a Niphiodicercaria; remainder without trematode parasites.
<i>Buynia</i> sp., prox. <i>inconspicua</i> , Dohrn	Village pond, Bolarum, Secunderabad.	5-ix	17	1-xi	4	No trematode parasites.
<i>Vivipara bengalensis</i> (Lamarck)	Tank, Indian Museum, Calcutta.	27-ix	30	3-xii	24	Do.
<i>Vivipara bengalensis</i> , var.	Belgatchia, near Calcutta	11-x	30	3-xii	29	Do.
<i>Pachylabra globosa</i> , Swainson	Tank, Indian Museum, Calcutta	3-x	14	10, 11-xi	14	Do.
<i>Pachylabra carinata</i> , Swainson	Pond near railway station, Waltair.	18-ix	8	21-xi	1	Do.
<i>Limnaea amygdalum</i> , Troschel	Hosanasagar Tank, near Secunderabad.	10-ix	4	30-x	4	Do.
<i>Limnaea amygdalum</i> , var. <i>gracilior</i> , von Martens.	Tank, Indian Museum, Calcutta.	19-ix	13	2-xii	2	Do.
Do.	Do.	28-ix	30	3-xii	8	Do.
<i>Limnaea ovalis</i> , Gray	Do.	27, 28-ix	30	5-xii	4	Do.
<i>Limnaea succinea</i> var. <i>sordida</i> , Troschel.	Pond at Trimulgherry, Secunderabad.	9-ix	17	2-xi	2	Do.
<i>Planorbis exustus</i> , Dehayes	Village pond, Bolarum, Secunderabad.	6-ix	20	30-x	15	Do.
Do.	Tank, Indian Museum, Calcutta.	23-ix	30	22-xi	14	Do.

TABLE I.

Showing the natural infection of certain molluscs with larval trematodes.

Species.	Locality.	Date.	Total number examined.	Number without trematode parasites.	Number with trematode parasites.	NUMBER WITH TREMATODE PARASITES.												
						Large Monostome.	Small Monostome.	Amphistome.	Gymnocephalous with eye-spots.	Gymnocephalous without eye-spots.	Megasturus.	Xiphidocerariae.	Furocercous.	Cercariae.	MIXED INFECTIONS.			
															Large Monostome and Xiphidocerariae.	Xiphidocerariae and Furocercous.	Indeterminable rediae or sporozoites.	
		1918																
<i>Melania<sup>1</sup> tuberculata</i> (Müller).	Hislop War Hospital, Trimulgherry, Secunderabad.	30-viii	50	21	29	25	..	..	..	..	..	2	..	..	1	1	..	..
Do.	Pond near railway station, Waltair.	19-ix	13	1	12	12	..	..	..	..	..	..	..	..	..	..	..	..
Do.	Basin in fern-house, Indian Museum, Calcutta.	10-x	20	13	7	4	3	..	..	..	..	..	..	..	..	..	..	..
<i>Melania variabilis</i> , Benson.	Tank, Indian Museum, Calcutta.	20-24-ix	85	62	23	1	..	..	..	..	..	..	..	22	..	..	..	..
<i>Melania lineata</i> , Gray	Do.	24-30-ix	60	9	51	2	43	..	..	..	6	..	..	..	..	..	..	..
<i>Amnicola arcuata</i> (Benson)	Pond near railway station, Waltair.	18-ix	31	31	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Do.	Tank, Indian Museum, Calcutta.	23-ix	42	41	1	..	..	..	..	..	..	1	..	..	..	..	..	..
<i>Bithynia</i> sp., prox. <i>inconspicua</i> , Dohrn.	Village pond, Bolarum, Secunderabad.	3-ix	50	43	7	2	..	..	3	1	..	..	..	..	..	..	..	1
<i>Vivipara bengalensis</i> (Lamarck).	Tank, Indian Museum, Calcutta.	28-ix	50	50	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>Vivipara bengalensis</i> , var.	Belgatchia, near Calcutta.	10-12-x	50	50	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>Pachylabris globosa</i> , Swainson.	Tank, Indian Museum, Calcutta.	3,14-x	9	9	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>Pachylabris carinata</i> , Swainson.	Pond near railway station, Waltair.	14,15-ix	63	54	9	..	..	..	..	9	..	..	..	..	..	..	..	..
<i>Limnaea amygdalum</i> , Troschel.	Hosanasagar Tank, near Secunderabad.	9-ix	15	15	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>Limnaea amygdalum</i> , var. <i>gracilior</i> , von Martens.	Tank, Indian Museum, Calcutta.	20-30-ix	50	50	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>Limnaea ovalis</i> , Gray	Do.	20-30-ix	50	50	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>Limnaea succinea</i> , var. <i>sordida</i> .	Pond at Trimulgherry, Secunderabad.	9-ix	26	22	4	..	..	..	..	..	1	..	..	..	..	..	..	3
<i>Planorbis exustus</i> , Deshayes.	Village pond, Bolarum, Secunderabad.	2-ix	50	44	6	..	..	4	..	..	..	..	2	..	..	..	..	..
Do.	Pond at Trimulgherry, Secunderabad.	9-ix	25	25	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Do.	Pond near railway station, Waltair.	16-ix	12	12	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Do.	Tank, Indian Museum, Calcutta.	Aug.	146	112	34	..	..	..	..	..	..	34	..	..	..	..	..	..
Do.	Do. <sup>2</sup>	6-x	17	17	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Do.	Rice-fields, Balasagon, Orissa. <sup>3</sup>	31-viii 1-ix	14	14	..	..	..	..	..	..	..	..	..	..	..	..	..	..

<sup>1</sup> The Indian species of Melaniidae or Lymnaeidae are left provisionally in the genus *Melania*, but this genus will certainly have to be divided on anatomical grounds. N. A.<sup>2</sup> Twenty individuals of medium or large size. Fifty small specimens were found<sup>3</sup> to be without parasites.<sup>3</sup> Examined by Dr. Baini Prasad.

## **NOTICE.**

# INDIAN SCIENCE CONGRESS

TO BE HELD AT NAGPUR

January 1920.

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### MEDICAL RESEARCH SECTION.

*The President of the section wishes to invite the attention of members who propose to attend the Medical Research Section to the following points :—*

#### I.

##### TYPES OF PAPERS DESIRED.

- (1) Announcing in general terms, with no unnecessary detail of description and without complex graphs and lists of figures, any new work performed by the author in any line which has a direct bearing on the advance of medical science and the prevention or treatment of disease.
- (2) Describing, illustrating and demonstrating new methods and technique.
- (3) Briefly summarising present knowledge and indicating promising lines of advance.
- (4) Opening up a discussion on important current medical problems about which there is, or may be, room for divergent opinions.
- (5) Dealing with diseases in animals and plants in so far as they have a bearing on human maladies.

##### ILLUSTRATIONS.

Illustrations add greatly to the interest and comprehensibility of a paper, but they should be simple enough or clear enough to be taken

in at a glance. Complex diagrams which require close inspection and study divert the attention of members from the text of the reader. Clear lantern slides are about the best form of illustration and are suitable for photographs, photomicrographs, graphs and short lists of figures. Line diagrams on a large scale on paper which can be pinned on a screen are also useful. Blackboard sketching or figuring is not recommended.

## II.

### TYPES OF PAPERS NOT ADMITTED.

- (1) Very detailed and complex, whether words or figures are involved.
- (2) Very special, which are outside the range of knowledge of those likely to be present.
- (3) Very general, which contain nothing new and put nothing in a new light.
- (4) Papers which would appeal more to other sections such as the entomological, chemical or botanical.
- (5) Papers on general or clinical medicine, surgery or therapeutics which would be acceptable at a general medical congress.

## III.

### MEMORANDUM.

- (1) No paper should occupy more than 15 minutes in the reading.
- (2) Authors should read their papers over beforehand to the clock to make sure that they can be delivered without haste within the specified time.
- (3) Authors should bear in mind that papers which are indistinctly or too rapidly delivered fail either to interest or instruct their audience.
- (4) Members attend in order to be interested and instructed. Minute details, tables of figures and complex graphs which cannot be easily followed by the audience merely bore them.
- (5) Papers read at the congress should be written for the congress and not as if for publication in a scientific journal. When a reader has in front of him a lengthy and detailed manuscript, which cannot possibly be read through in the time allotted to him, and tries to make excerpts from it and is



continually referring backwards and forwards and losing his place and the thread of his discourse, he fails to interest his audience.

- (6) Papers which are suitable for publication in appropriate journals may be either in length, matter or method of exposition quite unsuitable for presentation to the congress.
- (7) When members are invited to discuss or remark upon a paper which has just been read, they should confine their remarks strictly to the subject before the section and refrain from introducing irrelevant matter including personalities.
- (8) Legitimate discussion is confined within the following limits :—
  - (a) the production of additional evidence in favour of statements made or suggestion of means to obtain it ;
  - (b) the production of additional evidence throwing doubt on or controverting the statements made ;
  - (c) the questioning of the soundness of the methods used to obtain the results presented, and the suggestion of alternative or sounder methods ;
  - (d) asking for the elucidation by the author of obscure passages ;
  - (e) supporting or questioning the validity of arguments used ;
  - (f) supporting or questioning the conclusions drawn from the evidence presented ;
  - (g) supplying possible answers to questions raised ;
  - (h) raising questions relevant to the subject but not suggested by the author ;
  - (i) the presentation of a rival hypothesis.

Finally the author of the paper is given an opportunity of answering all criticisms and summarising the discussion. No other member may speak more than once except to correct misinterpretation of anything he may have said.



# THE PATHOGENESIS OF DEFICIENCY DISEASE.

No. V. HISTO-PATHOLOGY.

BY

BREVET-LIEUTENANT-COLONEL ROBERT McCARRISON,

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[Received for publication, August 11, 1919.]

THE present paper deals with the histo-pathological changes in—

The spleen,  
,, liver,  
,, pancreas,  
,, kidneys,  
,, pituitary body, and  
,, thyroids

of *pigeons fed exclusively on a diet of autoclaved milled rice*. It is a continuation of a previous report on the 'Pathogenesis of Deficiency Disease,' published in vol. VI, no. 3, pp. 275–355, of this Journal, and is to be read in connexion therewith. The histo-pathological changes in the intestines<sup>(2, 3)</sup> and testicles<sup>(1)</sup> of pigeons so fed have already been described.

## *The Spleen.*

This organ is greatly atrophied. I am aware of no other morbid process in which such pronounced atrophy of the spleen occurs.

The histological changes consist in—

- (1) thickening of the capsule and contraction of the organ;
- (2) atrophy of splenic pulp and partial disappearance of lymphoid cells;

- (3) increased deposit of pigment ; and
- (4) thickening of the walls of the arteries.

These make up a picture more remarkable than that presented by any other organ of the body, with the exception of the thymus, the testicles<sup>(1)</sup>, and the intestines. They are illustrated in Figs. 1-4.

In consequence of the atrophy of the splenic pulp and the reduction in numbers of the lymphoid elements of the organ, its fibrous trabeculae and arterial system stand out in sections with abnormal prominence. The capsule is much thickened and the organ contracted, both changes resembling closely those which occur in the testicles<sup>(1)</sup>.

The pigment in the atrophic spleen appears to be increased in amount. It is possible that its increase may be only relative, the same quantity being collected into the smaller compass provided by the contracted organ. It seems probable, however, that the increased blood destruction<sup>(1)</sup>, which occurs in consequence of the deficient diet, may to some extent be responsible for the increased deposit of pigment.

The lymphoid cells and the cells of the pulp are involved in a process of necrobiosis and absorption; they are greatly reduced in numbers. The nuclei lose their staining characters, a gradual karyolysis occurs and the cytoplasm ultimately disintegrates (Figs. 3 and 4). In the more atrophic spleens, only traces of Malpighian corpuscles are to be found.

The vessels are involved in a process of proliferative arteritis, often leading to great thickening of their walls and to constriction of their lumen (Figs. 3 and 4).

Since in pigeons the disappearance of the cellular elements of the spleen exceeds that occurring in any other organ of the body, with the exception of the thymus and the testicles, it seems probable that its cells are utilized for purposes of nutrition of more vital organs. The thickening of the vessels suggests that the pathological changes may in some part be due to the prolonged action of toxic substances generated in the course of a highly abnormal metabolism.

It is obvious that the functional capacity of the spleen is greatly impaired by the pathological changes which result in consequence of the deficient diet.

#### *The Liver.*

This organ undergoes atrophy; the atrophy, as determined by weight, is to a great extent masked by the congestion which usually accompanies it.

270<sup>b</sup>

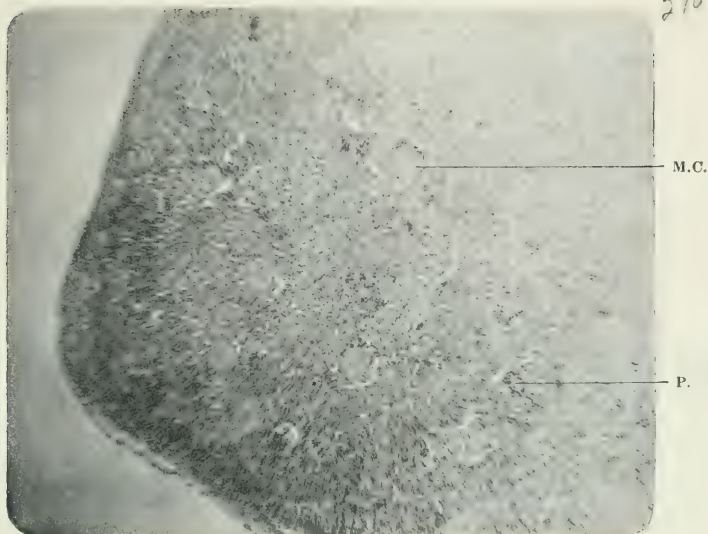


Fig. 1.—Spleen. Normal pigeon.

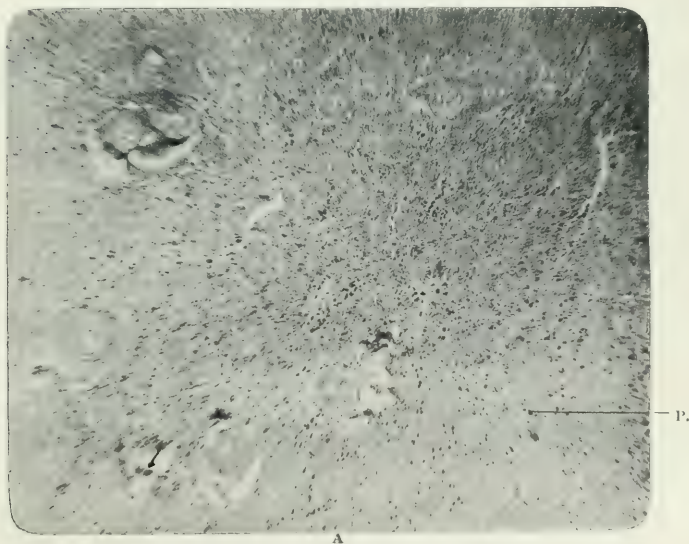
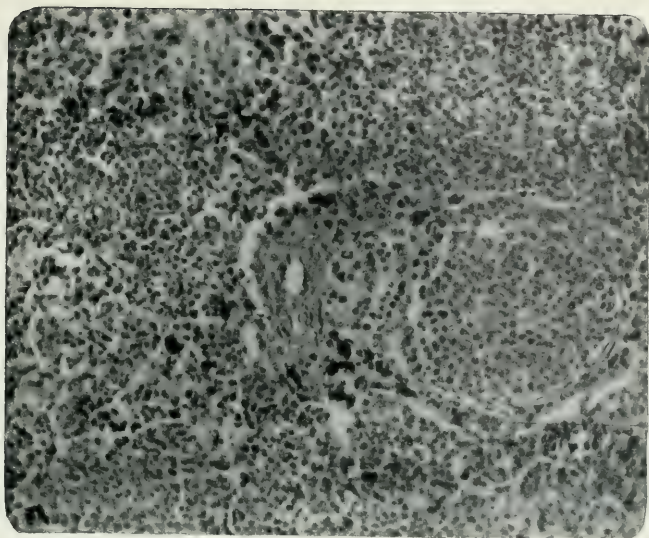


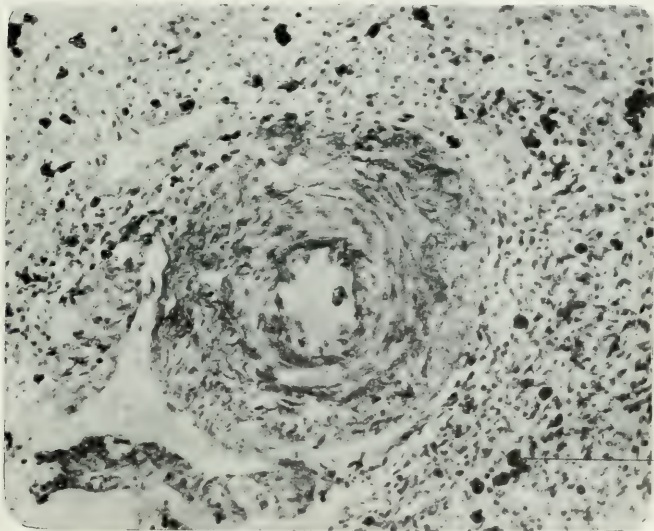
Fig. 2.—Spleen. Polyneuritis avium.





P.

Fig. 3.—Spleen. Normal pigeon.



P.

FIG. 4.—Spleen. Polyneuritis avium.





The histological changes consist in—

- (1) necrobiosis of the liver cells with karyolysis,
- (2) necrosis in cases complicated by infection, and
- (3) passive congestion.

The necrobiosis has no definite focal distribution; it affects single cells or small groups of cells. It varies in degree within wide limits in livers from different cases and in different parts of the same organ. The number of liver cells showing normally staining nuclei may not exceed one-tenth to one-fifth of the total cells of the organ.\* I have encountered several cases in which the number was considerably less. Cells containing nuclei which have lost in great measure their staining characters, owing to partial disappearance of the nuclear chromatin, may constitute as many as three-fifths of the total liver cells; in many such cells, in hæmatoxylin-stained sections, the nuclei could only just be distinguished under high powers of the microscope as faint rings empty of chromatin. Cells from which the nuclei have wholly disappeared may number one-fifth to one-third of the total cells of the organ; in exceptional cases their numbers have been much higher. The cell-body becomes homogeneous or the cell-wall contains but a granular debris. These appearances are illustrated in Fig. 5.

Extensive and more rapid death of cells occurs in cases where avian beriberi is associated with septicæmic states. In such cases necrotic changes in the liver cells may be very pronounced, the degenerated cells being separated one from another and from their basal attachments. In such septicæmic cases bacteria were sometimes seen, in sections of the organ, lying amongst the liver cells and were readily detected in smears, from its cut surface.

Both necrobiosis and necrosis are usually associated with greater or lesser degrees of passive congestion. The vessels are engorged and a fairly uniformly distributed hæmorrhagic infiltration may occur amongst the liver cells. Fig. 5 represents a moderate degree of congestive and necrobiotic change. In the graver cases of necrosis associated with septicæmia, the degree of congestion and of hæmorrhagic infiltration may be extreme and the liver be greatly enlarged in consequence.

I interpret these changes as indicating a gradual starvation of the cells, the process of necrobiosis and karyolysis being aided by passive congestion of the organ. It is possible that toxic substances produced

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\* Delafield's, Heidenhein's and Mann's stains were used.—R.M.CC.

in the course of a deranged metabolism or absorbed through the debilitated intestinal mucosa may contribute to the result ; malnutrition of the cells with failure of regeneration is, I believe, the essential factor concerned.

#### *The Pancreas.*

As estimated by weight, this organ undergoes considerable atrophy. In contrast, however, to the constant and fairly definite loss in weight, the histological changes in the pancreas are both inconstant and indefinite. In general its texture is less open in pigeons dying of avian beriberi ; the alveoli are often more closely approximated one to another ; the cells are smaller in size and appear to be functionally less active, their nuclei are more irregular in outline and more indefinite in staining characters, while alveolar cells from which the nuclei have disappeared are not uncommonly met with. Having regard to the normal processes of degeneration and renewal of cells which go on in the pancreas in health, the histological appearances presented by this organ differed little from those of health in seven cases out of twenty-four examined. Fig. 6 illustrates such a case. This specimen is from a pigeon dying of typical avian beriberi with pronounced cerebellar symptoms. The loose alveolar structure was well preserved while only a small number of the polyhedral alveolar cells exhibited necrobiosis and karyolysis. Fig. 7, on the other hand, although taken from a similar case, shows a different structure ; the organ is of close texture, the alveolar cells smaller and the nuclei are area for area more abundant than in health.

I have been unable to satisfy myself that the islets of Langerhans are more prominent in the pancreas from cases of avian beriberi than in health, although the state of chronic inanition which accompanies this malady would lead one to expect a greater prominence of islets. Nor have I noted a greater tendency to degenerative change in the islets than in other cellular elements of the organ as a whole.

The necrobiosis of alveolar cells is usually limited to single cells or to small groups of cells ; sometimes, however, it involves larger areas, especially at the periphery of the gland (Fig. 7). On the other hand, the number of the nuclear elements may, in some cases, or in certain parts of the section, show an excess over that found in health. This appearance is usually due to shrinkage of the polyhedral cells of the alveoli and of the alveoli themselves (compare Figs. 6 and 7). Collections of round cells usually at the periphery of the gland are occasionally encountered, and a

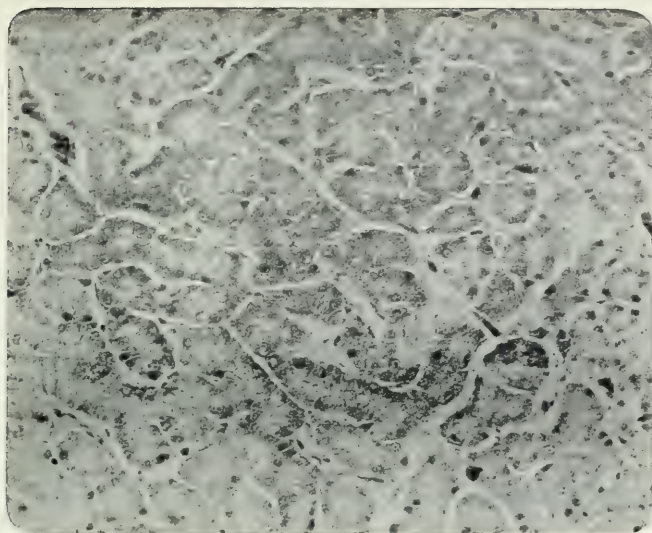


Fig. 6.—Pancreas. Polyneuritis avium.

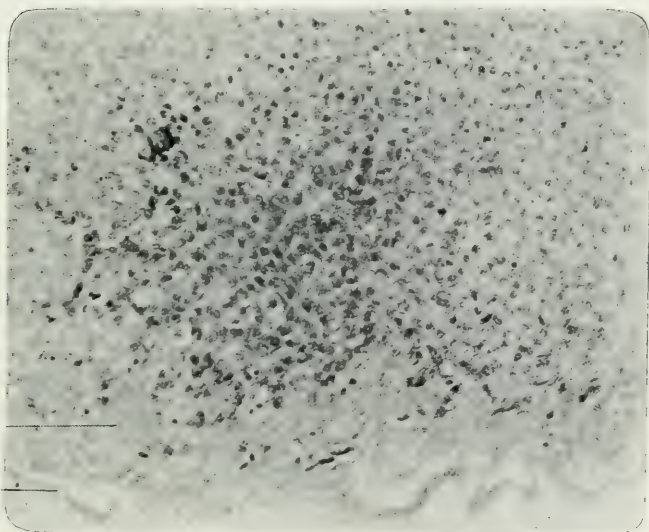


Fig. 7.—Pancreas. Polyneuritis avium.



relative increase of connective tissue elements especially at its periphery has sometimes been noted. These areas of peripheral necrosis probably correspond to the necrotic-like areas sometimes seen on the surface of the organ by the naked-eye<sup>(1)</sup>.

Varying degrees of congestion and hæmorrhagic infiltration are not infrequent. In some few cases the hæmorrhagic infiltration has been present in extreme degree—an appearance which is in conformity with the naked-eye evidences of congestion seen in some cases<sup>(1)</sup>. A thickening of the arterial walls comparable to, but much less marked than, that seen in the spleen has occasionally been noted; in general, however, there is no characteristic or constant change in the vessels.

With the exception of a few cases in which intense hæmorrhagic infiltration of the organ had occurred, or in which peripheral necrosis was marked, the pathological changes in the pancreas were not such as to cause great derangement of its function, although in the majority they were undoubtedly sufficient to impair to some extent its functional capacity. To what extent the chemical activities of the organ may be disordered by the deficient dietary I am unable to say. In this connexion, however, it is to be noted that vitaminic extracts prepared from brewer's yeast cause, on intravenous injection, an increased flow of pancreatic secretion, their action in this regard being similar to that of secretin<sup>(5)</sup>. I have noted in one case of gastro-intestinal debility, in the human subject, a decided improvement in pancreatic function, as well as of biliary function, following on the oral administration of a vitaminic extract prepared from the yolks of eggs; the gastro-intestinal disorder was traceable in this case to subsistence for many years on a diet consisting largely of bread-and-butter and milk puddings.

### *The Kidneys.*

These organs lose little or no weight in consequence of the deficient dietary.

The histological changes consist in—

- (1) congestion, and
- (2) cellular changes.

Some degree of congestion is an almost constant feature. It is sometimes slight and confined to engorgement of the vessels, but more often it involves the glomerular tufts or gives rise to hæmorrhagic extravasations of greater or lesser degree between the tubules (Figs. 10 and 11).

The cells of the organ may exhibit little or no change, or slight degrees of cloudy swelling may be present. Usually there is no marked degree of necrobiosis, but occasionally specimens are met with in which the cells have undergone extensive necrosis. I have not found that cases presenting evidences of oedema are to be distinguished by the greater severity of cellular change in the kidney. In general the changes in the organ are not so severe as to impair greatly its functional capacity. Cases, however, are occasionally encountered in which the pathological changes are such as would cause disturbance of renal function.

#### *The Pituitary.*

The glandular part of this organ tends to increase slightly in weight in consequence of the deficient dietary. Moderate congestion is the main histological feature presented by this part of the pituitary body. In healthy pigeons the gland frequently shows minute acini containing droplets of colloid-like material. In cases of avian beriberi, these colloid accumulations are in general less frequently seen, although they are by no means constantly absent. The vessels of the organ are usually engorged and some degree of hæmorrhagic infiltration between the secreting cells is frequently present. The slight increase in weight is, I think, due to the greater content of blood. In some cases the nuclear elements are present in greater numbers in individual fields of the microscope than in health, but I have been unable to find evidences of nuclear division. Figures 8 and 9, in which the thickness of the sections and their magnification are the same, illustrate these points. Evidences of karyolysis or karyorrhexis are occasionally encountered, but in general nuclear changes are scanty. The histological changes in this part of the pituitary body are comparable in kind and in degree to those found in the thyroid and constitute but a slight departure from the histological appearances of health.

The *pars nervosa* of the pituitary body has not been examined in pigeons.

#### *The Thyroids.*

These organs lose slightly in weight in consequence of the deficient dietary.

Owing to the great variations in histological appearances of the thyroid in health it is impossible to form an accurate estimate of the degree of histological change in avian beriberi from the microscopical



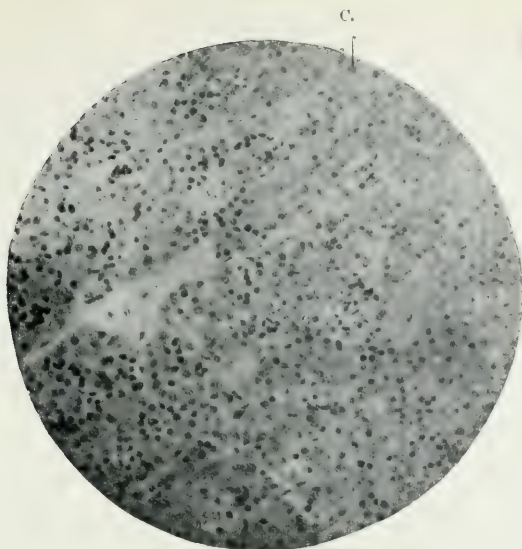


Fig. 8.—Pituitary. Normal pigeon.

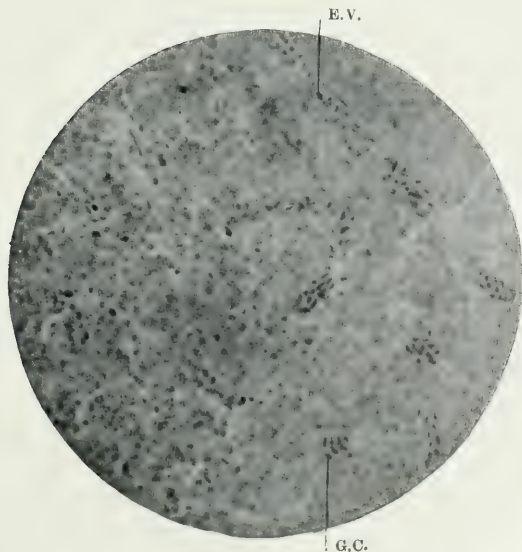


Fig. 9.—Pituitary. Polyneuritis avium



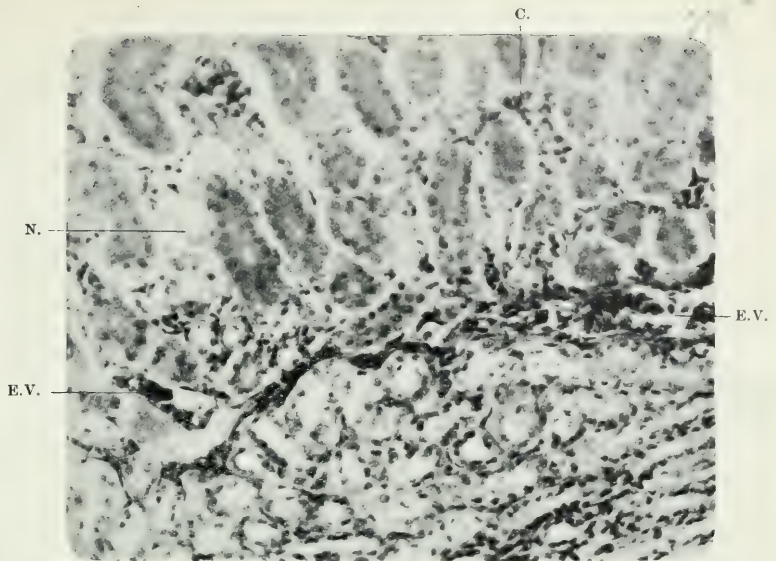


Fig. 10.—Kidney. Polyneuritis avium.  
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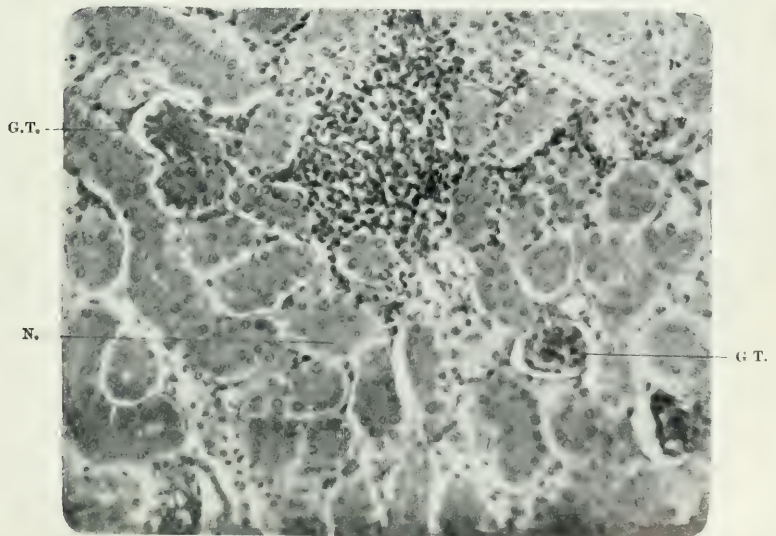


Fig. 11.—Kidney. Polyneuritis avium.



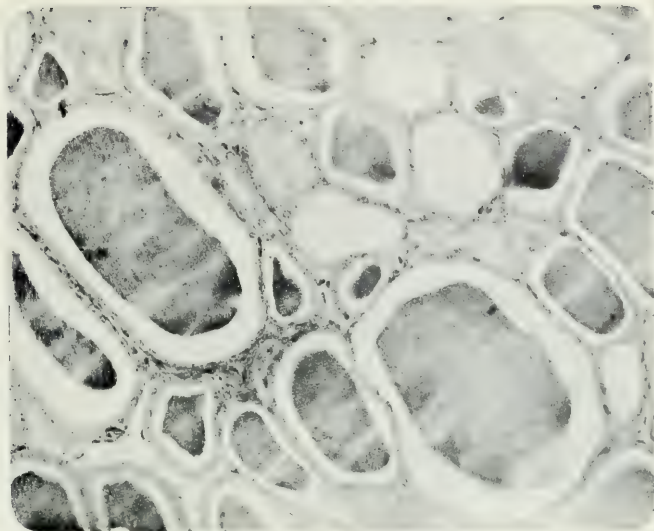


Fig. 12.—Thyroid. Polyneuritis avium.

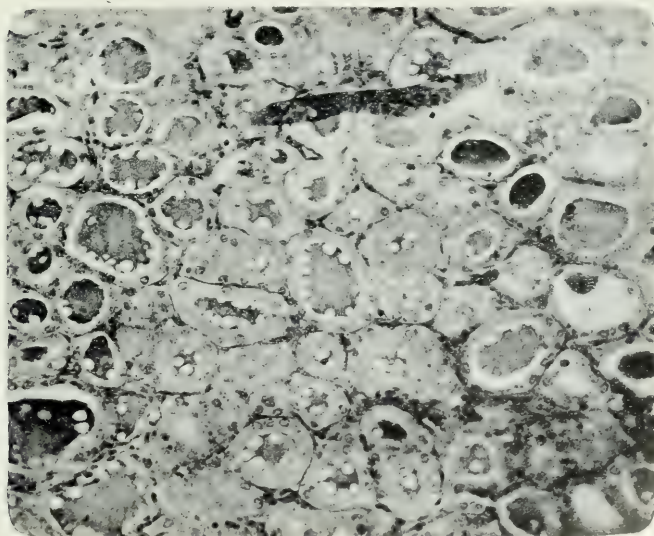


Fig. 13.—Thyroid. Normal pigeon.



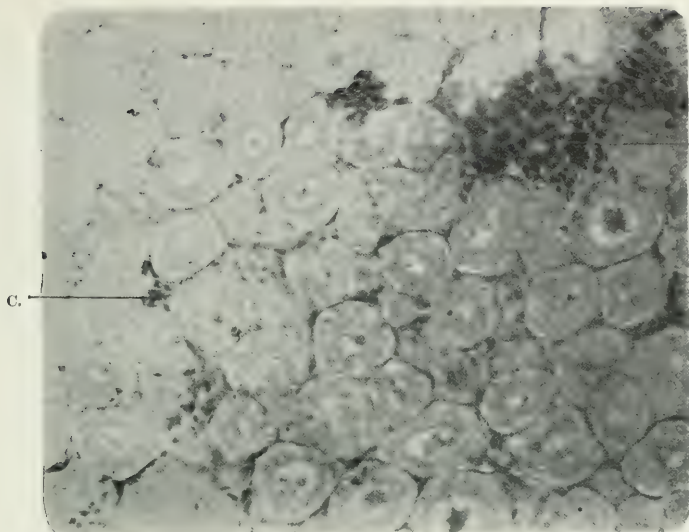


Fig. 14.—Thyroid. Polyneuritis avium.

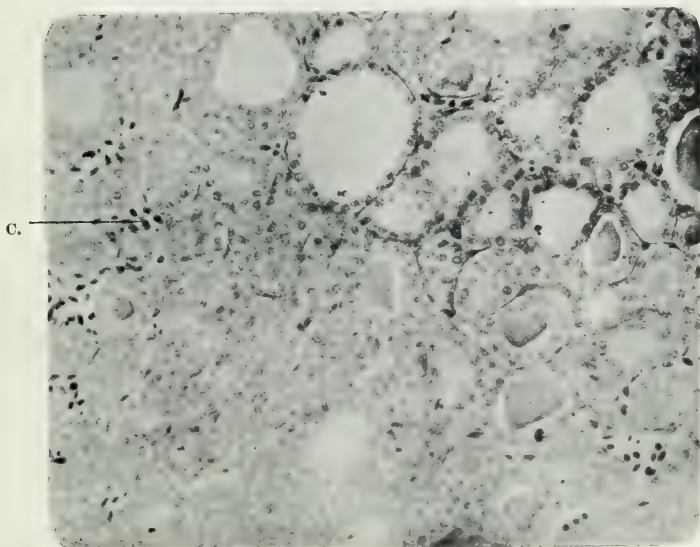


Fig. 15.—Thyroid. Polyneuritis avium.





examination of a small number. The present report is based on an examination of serial sections of the thyroids from thirty cases of avian beriberi and from thirty healthy pigeons.

In healthy and non-goitrous pigeons in this locality, 6,000 feet above sea-level, the thyroids fall into two main groups and into variants of these: (1) colloid or resting glands, and (2) actively secreting glands<sup>(4)</sup>. Between these two extremes, exemplified in Figs. 12 and 13, many different appearances are seen according as the organ is assuming the phase of active secretion from the colloid or resting state, or reverting from the phase of active secretion to the colloid or resting state. In regard to the phases of the normal thyroid glands' activity, these organs in pigeons differ in no respect from those of mammals in which I have hitherto studied them<sup>(4)</sup>.

In the thirty healthy pigeons examined, rather more than one-half had thyroids of the colloid or resting class (Fig. 12); the remainder came within the category of actively secreting glands (Fig. 13) or of glands reverting to the colloid state.

Excluding one case in which enlargement of the thyroids, amounting to actual goitre, occurred, these organs, in thirty cases of experimentally-produced avian beriberi, fell into four main groups: (1) colloid glands—nine, (2) actively secreting glands—seven, (3) reverting glands—nine, and (4) 'infected' glands—five.

*Colloid glands* (Fig. 12) were less often met with in cases of avian beriberi than in health. Under low powers of the microscope they differed little or not at all from similar colloid glands from healthy birds. Higher magnification of the acinar epithelium sometimes revealed cells in which necrobiotic changes had undoubtedly occurred, constituting interruptions in the continuity of the uniform row of nucleated cells lining the acini. Having regard, however, to the fact that degeneration and renewal of acinar cells is a normal process in the thyroid gland, such appearances cannot be considered abnormal unless they are excessive. In general the evidences of departure from normal were scanty, and in some cases they were actually wanting. The colloid substance appeared not to differ histologically from normal.

Thyroids falling within the category of '*actively secreting glands*' comprised less than one-fourth of the cases examined. In some of these the vascular connective tissue envelope surrounding the acini appeared more prominent than in health, moderate degrees of congestion being usually present (Fig. 14).

In general the nuclei of the acinar cells stained less clearly, an abnormal proportion showing karyolysis. Apart, however, from slight congestion, several thyroids were met with in this category which I was unable to distinguish from healthy glands.

Thyroids in the stage of reversion to or from the colloid state made up the major part of the remaining cases (Fig. 15). In these a moderate or slight degree of congestion was the most constant abnormal feature. In some cases evidence of necrobiotic change was present in a proportion of the acinar cells; but in this category also thyroids were encountered which differed little or not at all from the glands of healthy pigeons.

The thyroid glands were examined in five cases in which beriberi was associated with a septicæmia; in these cases the thyroids invariably came within the category of 'infected' glands. The changes seen in such glands are distinctive and pronounced: the organ is congested in marked degree; the alveolar cells are necrotic and separated from one another and from their basal attachment; disintegrating cells occupy the acini which, owing to denudation of their epithelial lining, are often obscure in outline; colloid is scanty, absent, or existing as isolated acidophile globules lying free amongst the necrotic and necrosing cells. I have found it possible, with only a small percentage of error, to distinguish between simple avian beriberi and that associated with septicæmia by the histological appearances of the thyroids alone.

Excluding then infective processes, which so constantly reveal their presence in the body by initiating histological changes in the thyroid gland, I find this organ to be amongst the least affected of all organs by the deficient dietary. The changes which are attributable to this cause consist in mild or moderate degrees of congestion and in necrobiosis of a relatively small proportion of the secreting cells.

Confusion as to the histological changes directly attributable to the dietetic deficiency is apt to occur unless large numbers are examined and unless complicating infections are excluded. It is to these that pronounced congestion, necrosis and denudation of the alveolar epithelium are mainly due and not, as I had previously concluded<sup>(4)</sup>, to the dietetic deficiency. To what extent the chemical activities of the thyroid gland may be altered by the deficient dietary I am unable to say.

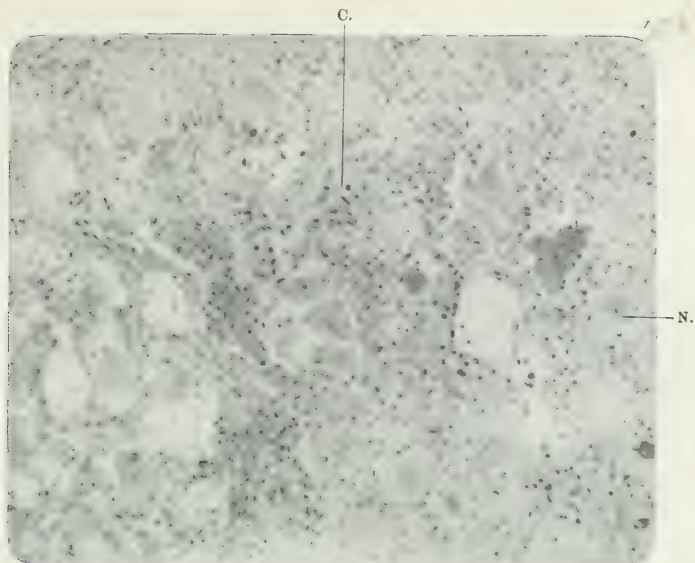


Fig. 16.—Thyroid. Polyneuritis avium.



Fig. 5.—Liver. Polyneuritis avium.



*The Intestines.*

In another paper<sup>(3)</sup> I have described in detail the changes found to result in the intestines of pigeons from an exclusive diet of autoclaved rice. These changes consist in (1) congestion and hæmorrhagic infiltration, and in (2) atrophic, necrotic and more rarely inflammatory changes in the tissue elements composing the coats of the bowel. They are referred to again merely to draw attention to their conformity with those found in other organs of the body.

*The Thymus.*

With regard to this organ, atrophy is usually so extreme as to leave nothing for histological examination but the fibrous framework of the organ.

## SUMMARY.

In general, the histo-pathological changes found in uncomplicated cases in the various organs here dealt with are of two kinds: (1) necrobiosis, and (2) congestion. In some organs—the thymus, the spleen, and the testicles—the disappearance of tissue cells is the more prominent change. In others—the intestines, the liver, the pancreas, and the kidneys—congestion, with hæmorrhagic infiltration, is often the more prominent feature, although there are exceptions to this generalization. In others again—the thyroids and the glandular part of the pituitary—both features may be present but usually in mild degree.

The hæmorrhagic changes are the result in pigeons of passive congestion and, it may be also, of deficiency of anti-scorbutic factors in the food; the necrobiotic changes are in the main the result of malnutrition of the cells and more especially, I think, of nuclear starvation. Toxic irritation consequent, it may be presumed, on abnormal metabolic processes or on the action of ferments generated from necrobiotic cells may possibly contribute to them.

In the order of severity in which these changes occur, the organs may be ranged as follows:—

The thymus,  
,, spleen,  
,, testicles,  
,, intestines,  
,, liver,  
,, kidneys,  
,, pancreas,  
,, thyroids, and  
,, pituitary.

The extraordinary hypertrophy of the adrenal glands observed in pigeons fed exclusively on autoclaved rice will be considered separately.

In general, then, the organs which suffer most are those which are least essential to the life of the individual. Next in order are the organs of digestion and assimilation, then the organs of excretion, and, lastly, the organs of internal secretion. That the central nervous system suffers least, from the point of view of organic lesion, is shown by the rapidity with which the nervous symptoms, due to the deficient diet, can be controlled or abated by the administration of vitaminic substances. It seems probable that the cellular elements of the organs least essential to the life of the individual are utilized to provide accessory food factors and other nutritive materials for the cells of higher function.

#### CONCLUSIONS.

(1) An exclusive diet of milled and autoclaved rice gives rise in pigeons to atrophic and congestive changes in the thymus, the spleen, the testicles, the intestines, the liver, the pancreas, the kidneys, the glandular part of the pituitary and the thyroids.

(2) These changes are most marked in the organs least essential to the life of the individual—the thymus, the testicles, and the spleen; in these atrophy is the predominant feature.

(3) The atrophic and congestive processes gravely affect the organs of digestion and assimilation and to a less extent the kidneys.

(4) The thyroid and the glandular part of the pituitary are less affected than are other organs of the body.

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- |     |                                |    |        |   |
|-----|--------------------------------|----|--------|---|
| (1) | McCARRISON, R.                 | .. | (1919) | <i>The Pathogenesis of Deficiency Disease, I. Indian Journal of Medical Research, VI, 3, pp. 275-355.</i> |
| (2) | "                              | .. | ..     | (1919) <i>Indian Journal of Medical Research, VII, 2, this number.</i>                                    |
| (3) | "                              | .. | ..     | (1919) <i>Indian Journal of Medical Research, VII, 1, pp. 167-187.</i>                                    |
| (4) | "                              | .. | ..     | (1917) <i>The Thyroid Gland in Health and Disease: London.</i>  |
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# THE PATHOGENESIS OF DEFICIENCY DISEASE.

## NO. VI. THE INFLUENCE OF A SCORBUTIC DIET ON THE BLADDER.

BY

BREVET-LIEUTENANT-COLONEL ROBERT MCCARRISON,

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*(Pasteur Institute of Southern India, Coonoor.)*

[Received for publication, August 11, 1919.]

THE effects of a scorbutic diet on the bladder were studied in guinea-pigs. The animals, five in number, were fed on crushed cats and autoclaved milk. An equal number of controls were fed on crushed cats, autoclaved milk, and abundance of green vegetables.

A diet of crushed cats and autoclaved milk is deficient in accessory food factors of the 'C' class; it is probably also deficient in other substances essential for the maintenance of perfect health in guinea-pigs.

Those animals fed on the scorbutic diet died within periods varying from nineteen to twenty-nine days. Their average initial weight was 532 grams; the average loss of weight amounted to 152 grams. One only developed clinical signs of scurvy; this animal had survived the deficient dietary for twenty-nine days. In two only were *naked-eye* evidences of a scorbutic state obvious at autopsy; one of these survived the deficient dietary for twenty-nine days, the other for twenty-five days. In all, hæmorrhagic infiltration of the organs—the adrenals<sup>(2)</sup>, the kidneys, the liver, the intestines<sup>(1)</sup>—was present in greater or lesser degree on histological examination. The heart's blood

of the guinea-pig which exhibited clinical evidences of scurvy yielded on culture at autopsy a coliform organism. The adrenalin-content of the suprarenal glands in this animal was excessively low<sup>(2)</sup>.

#### CLINICAL EVIDENCES OF DERANGEMENT OF THE BLADDER.

Hæmaturia was observed in one animal only ; in this the clinical evidences of experimental scurvy were well marked. In the four remaining animals hæmaturia was not observed. The urine was not examined microscopically ; it seems probable, therefore, that a slight degree of hæmaturia may have occurred (*vide infra*) in one other case, although not detected clinically.

#### NAKED-EYE EVIDENCES OF DERANGEMENT OF THE BLADDER.

At autopsy the bladder was empty and tightly contracted in all cases. In two cases it resembled an acorn set in a thick-lipped cup. This appearance was due to swelling of the tissues around the neck of the bladder.

Engorgement of the vessels with sub-peritoneal ecchymoses was a prominent feature in four cases. In some the congestion of the organ was more marked at its apex, in others at its base. On opening the bladder and examining the mucous surface with the hand-lens, congestion of its mucous membrane, with patchy ecchymoses at various points, was present in greater or lesser degree in all cases. The congested and ecchymotic areas were of unequal distribution and of varying extent in different animals.

#### HISTOLOGICAL EVIDENCES OF DERANGEMENT OF THE BLADDER.

The histological changes consisted in :—

(1) congestion of all coats, with hæmorrhagic infiltration of the mucous membrane ; and

(2) degenerative changes in the epithelium of the mucous membrane.

*Hæmorrhagic infiltration.*—The vessels of the bladder walls were intensely engorged in four cases. Extravasation of blood corpuscles into the submucous and muscular coats and between the epithelial cells of the mucous membrane was an almost constant feature (compare Figs. 1 and 3). This extravasation was of varying degrees of intensity in different animals and at different parts of the bladder wall ; it was not present at every area of the mucous surface but was confined to

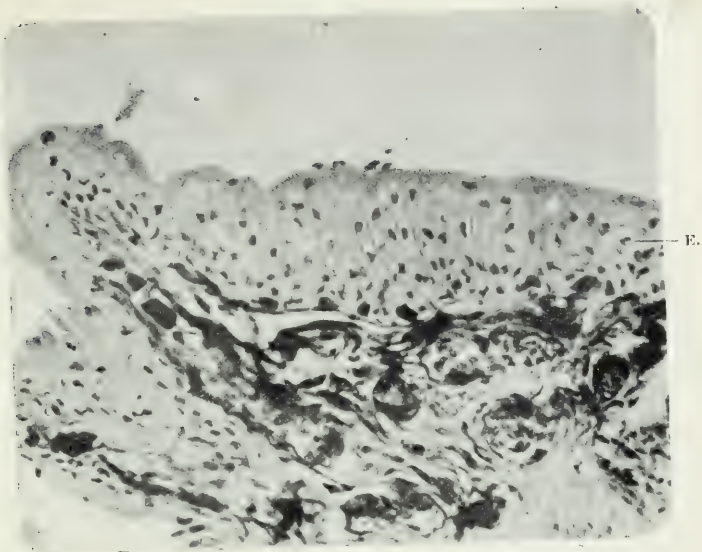


Fig. 1.

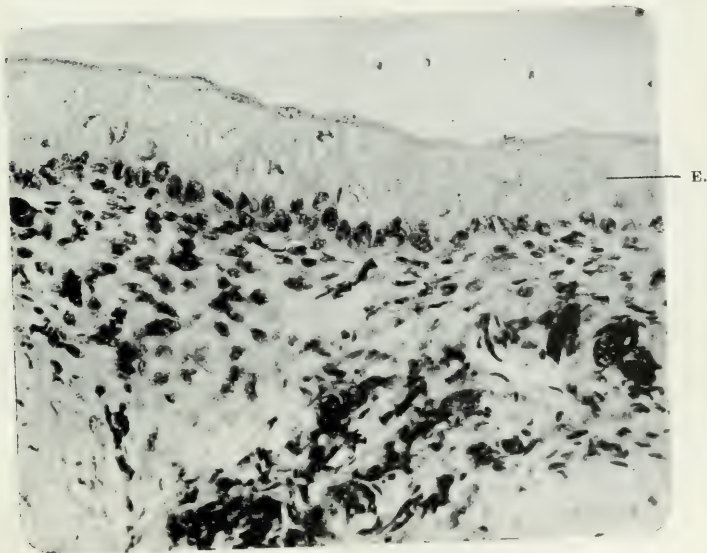


Fig. 2.



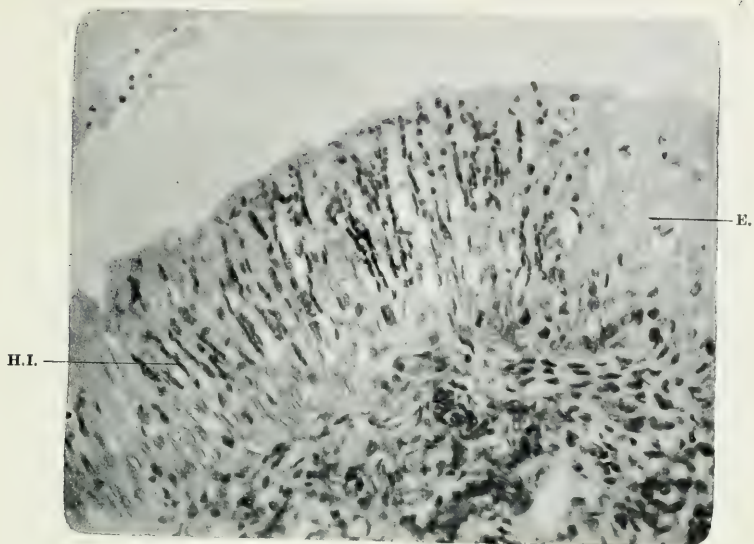


Fig. 3.

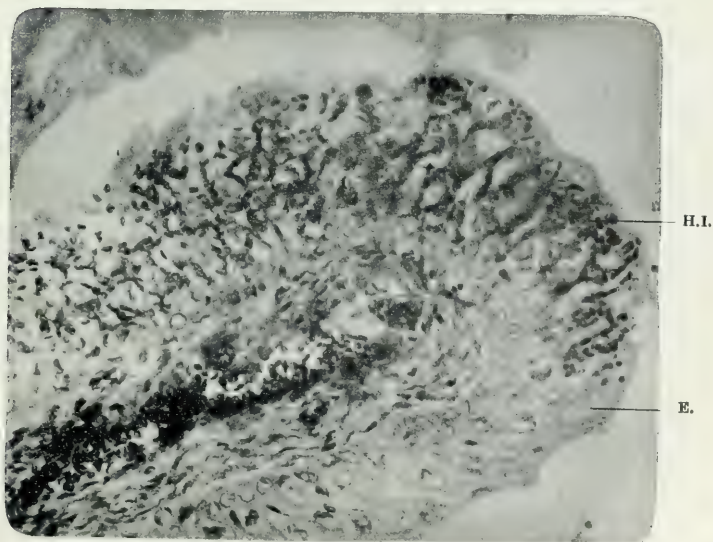


Fig. 4.



H.I

D.E.C.

E.

B.C.

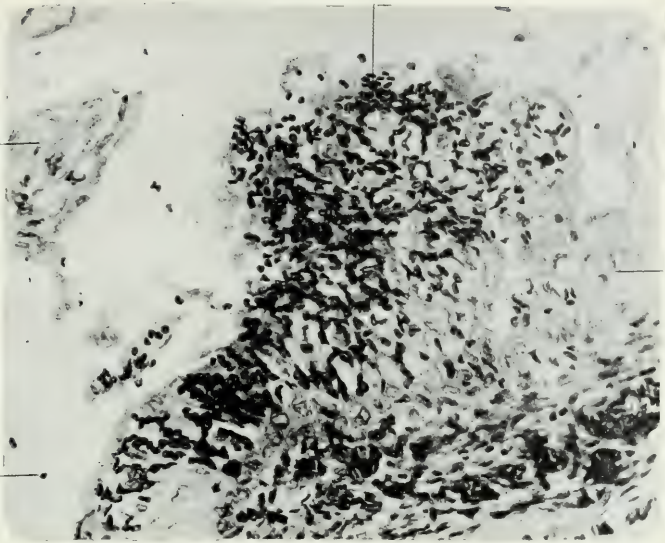


Fig. 5.

H.I.

H.

E.



Fig. 6.





certain points, the intervening areas showing little or none (Fig. 2). In one animal hæmorrhagic infiltration was found at only two points and was of lesser degree than that seen in Fig. 3. This figure typifies the appearances seen at several points of the lining epithelium in two other animals. Fig. 4 illustrates the degree of hæmorrhagic infiltration in a fourth animal, and Figs. 5 and 6 that found in the fifth, in which hæmaturia was a clinical feature of the case. In animals showing the lesser degrees of hæmorrhagic infiltration, the infiltrating corpuscles either did not reach the marginal layer of epithelial cells, or having reached it were extruded into the lumen of the viscus in but small numbers (Fig. 3). In the more pronounced degrees of hæmorrhagic infiltration, blood corpuscles were extruded into the cavity of the bladder in larger numbers (Fig. 4), or in such quantity as to constitute actual hæmorrhages (Figs. 5 and 6). It will be noted that the hæmorrhagic infiltration often caused the epithelial lining to bulge into the cavity of the bladder at the points where it occurred (Figs. 4 and 5). The various stages in the hæmorrhagic process are well shown in the photo-micrographs and call for no further description.

Congestion of the submucous and muscular coats was present in greater or lesser degree in all cases.

*Degenerative changes in the bladder epithelium.*—These consisted in—

- (1) swelling of the epithelial cells ;
- (2) swelling of the nuclei of these cells and loss of their staining characters ; and
- (3) desquamation of degenerated epithelial cells.

These changes are sufficiently well illustrated by the photo-micrographs ; they require no further description. Fig. 2 is introduced to illustrate an area of the bladder mucous membrane where no hæmorrhagic infiltration of the epithelial covering was present. In this the degenerative changes in the epithelial cells are well seen.

#### COMMENTARY.

Considered from the clinical point of view, these findings afford an explanation of the comparatively frequent occurrence of hæmaturia in human scurvy. They indicate that this symptom, which is usually a late manifestation of the scorbutic state in man, is the clinical evidence of an extreme degree of congestive and degenerative change in the

mucous coat and epithelial lining of the bladder. They indicate also that congestive states of the bladder may occur in guinea-pigs, fed on a scorbutic diet, which exhibit no obvious clinical evidences of scurvy during life, and little or no *naked-eye* pathological evidences of this malady after death.

Congestion of the bladder without clinical evidences of hæmaturia may then be regarded as a pre-scorbutic process in guinea-pigs. This being so, a point of practical importance to the physician is to ascertain, by clinical and therapeutical observation, whether congestion of the bladder may not be so produced in man and if so whether certain abnormalities of micturition may not sometimes be evidences of a pre-scorbutic state in human beings, especially in children.

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## THE PATHOGENESIS OF DEFICIENCY DISEASE.

### NO. VII. THE EFFECTS OF AUTOCLAVED RICE DIETARIES ON THE GASTRO-INTESTINAL TRACT OF MONKEYS.

BY

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THE experiment with which the present paper deals had for its object the production of beriberi in monkeys by means of dietaries of autoclaved rice.

For this purpose thirty-six young monkeys (*macacus sinicus*) were imported from Madras. The animals had recently been captured in the local jungles. With one exception, they were in perfect health. The exception occurred in the case of a monkey which died of general peritonitis one week after its arrival in Coonoor. Colonel Donovan, I.M.S., examined the peripheral blood in the majority of the animals; he found no blood parasites.

The monkeys were very wild and difficult to handle. After weighing, they were placed in separate cages and grouped as follows:

Group I: Control monkeys: twelve in number.

Group II: Monkeys fed exclusively on autoclaved rice: twelve in number.

Group III: Monkeys fed on autoclaved rice plus butter: eleven in number.

The diet of the control monkeys consisted at first of bananas, bread, milk and onions; monkey-nuts were subsequently added.

The milled rice, which formed the basal diet of Groups II and III, was picked by hand in order to remove as far as possible all grains having portions of adherent pericarp. It was then soaked for forty-eight hours

in several changes of water. Finally, it was autoclaved at a temperature of 130°C. for one hour-and-a-half.

The experiment commenced in the case of Groups I and II on 14th May, 1919. The animals were fed at 10 A.M. and again at 5 P.M. They were watered one hour before meals. It was found that those in Group II could not eat more than two ounces of autoclaved rice at each meal. Four ounces of autoclaved rice per diem was, therefore, fixed as the ration for the animals in Groups II and III.

In the case of Group III, the experiment commenced on 2nd June, 1919. Three pounds of autoclaved rice, prepared as above, were mixed by hand with 5½ ounces of fresh butter, giving a daily allowance of over four ounces of rice and half-an-ounce of butter for each animal. The morning and evening feeds thus consisted of two ounces of autoclaved rice and one-quarter ounce of fresh butter.

The average initial weight of the monkeys in Groups II and III was approximately two kilos; their daily allowance of rice (4 ozs.) was, on this basis, much greater than that included in the rice ration of Indian jails.

In Group II, the exclusive diet of autoclaved rice was lacking in all classes of accessory food factors; it was also deficient in suitable proteins, in fats and salts, and was excessively rich in starch. In the case of Group III, accessory food factors of the 'B' and 'C' classes were wanting, while the diet was still ill balanced with respect to proteins, carbohydrates and salts.

#### COURSE OF THE EXPERIMENT.

For the first seven to ten days the animals in Groups II and III maintained good, or moderately good, appetites; during the first week they ate greedily, during the second they ate with less zest and began to leave much of their ration untouched. Later they exhibited a loathing for the food and either ate sparingly or refused to eat. They crumbled up the block of autoclaved rice, as if searching for more nutritious particles; finding none they threw the mass from them. Pigeons, in their search for suitable grains among the autoclaved rice, exhibit the same instinct in picking up grain after grain only to discard them. Finally, the animals retreated to the corner of their cages refusing to eat.

At this stage it was realized that the experiment must fail in its original object, *viz.*, to produce beriberi, and that the factor of inanition must play a leading part in the production of the resultant morbid changes. Nevertheless the trial was persisted in, in the case of fourteen animals,

with the new object of observing the effects of malnutrition on animals closely allied to man. It is with these effects, in so far as they relate to the gastro-intestinal tract, that the present paper deals.

In the following table, the duration of the experiment, together with the initial and final weights of the animals, and the chief clinical manifestation of gastro-intestinal disorder exhibited by them, are shown :—

TABLE I.

No. of monkeys.	Sex	Original weight in grams.	Final weight in grams.	Loss of weight in grams.	Number of days under experiment.	Diet.	Clinical evidences of gastro-intestinal disorder.
1	Male	2,000	1,500	500	19	Autoclaved rice	Diarrhœa becoming dysenteric.
2	Female	1,350	950	400	26	Ditto.	Diarrhœa becoming dysenteric (amœbic).
3	Male	1,470	1,040	430	22	Ditto.	Diarrhœa.
4	Female	1,570	1,170	400	22	Ditto.	....
5	Male	1,900	1,400	500	24	Ditto.	Diarrhœa becoming dysenteric (amœbic).
6	Male	1,380	980	400	19	Ditto.	Ditto.
7	Female	1,930	1,450	480	26	Ditto.	Diarrhœa.
8	Female	1,650	1,320	330	26	Ditto.	Dysentery.
11	Male	1,900	1,470	430	22	Ditto.	....
12	Male	1,780	1,420	360	28	Ditto.	Diarrhœa.
27	Male	1,810	1,250	560	15	Autoclaved rice and butter.	Ditto.
29	Female	2,130	1,720	410	13	Ditto.	Dysentery (amœbic).
30	Female	2,520	1,850	670	14	Ditto.	Diarrhœa (sprue-like).
36	Male	2,410	1,800	610	18	Ditto.	Ditto.
13	Female	2,300	2,300	..	33	Normal food	Nil.
20	Male	2,470	2,420	50	40	Ditto.	Nil.

*Note.*—Although twelve control monkeys were kept under observation throughout the course of the experiment, two only were killed at its termination for purposes of comparison with those dying in consequence of the deficient diet. It was considered unnecessary to use more controls for this purpose.

## LOSS OF BODY-WEIGHT.

It will be observed from the preceding table that monkeys fed exclusively on autoclaved rice lost weight at the average rate of eighteen grams per diem. Those fed on autoclaved rice plus butter lost weight much more rapidly: 37·5 grams per diem, or twice as much as those receiving no butter. As the animals were not weighed daily but only at the commencement and at the end of the experiment, the daily averages given are of course approximate. It is probable that the loss of weight was much more rapid towards the close of the experiment than during its earlier days.

The animals which received butter also died much more rapidly than those which received no butter: the former in an average of 15 days, the latter in an average of 23·4 days.

The sex of the animal appears to have influenced the results to some extent; the figures are, however, too small to justify any definite conclusions on this point. In Group II the average loss of weight was greater and death occurred more rapidly in males than in females (1); in Group III the loss of weight was greater in males than in females although in the former death was longer delayed.

## CLINICAL EVIDENCES OF DISEASE.

No observations were made with regard to body-temperature and respiration as the monkeys were too wild to handle.

In addition to loss of body-weight, the main clinical evidences of disease exhibited by the animals in Groups II and III were—

- (1) progressive anæmia,
- (2) gastro-intestinal disorders, and
- (3) progressive asthenia ending in death.

No clinical evidences of polyneuritis or of œdema were observed in any: in one case (No. 7) 1 c.c. of fluid was found in the pericardial sac at autopsy.

The attitude assumed by the animals during the later days of the experiment was such as to suggest that they suffered much from headache; this was doubtless the case since marked congestion of the cerebral vessels was observed at autopsy in the majority of cases. They often sat crouched up in a corner of their cages with their heads in their hands or lay prone with the hand supporting the head. Both attitude and facial expression suggested extreme misery.



The animals became daily more anæmic ; the blanched face, lips and skin and the facial expression were in marked contrast to controls in neighbouring cages. Asthenia day by day became more pronounced ; from a state of alertness and healthy activity the animals soon lapsed into one of listlessness and became slow and sluggish in their movements. Soon they were too weak to stand, and later the sitting posture could not be maintained. Then the enfeebled body toppled over on its side remaining in this position until death occurred.

#### CLINICAL EVIDENCES OF GASTRO-INTESTINAL DISORDER.

Gastro-intestinal disorder was evidenced by—

- (1) loss of appetite,
- (2) vomiting,
- (3) diarrhœa, and
- (4) dysentery.

The *loss of appetite* was mainly due to loathing of the food. This loathing was accentuated by the gastro-intestinal derangements resulting from the deficient dietaries. The symptom is an important effect of vitaminic deficiency.

*Vomiting* was observed in four cases (*vide* Appendix) ; it probably occurred oftener as the animals could not be kept under constant observation. The symptom did not persist for more than two days ; the animals so affected refused all food and died rapidly.

*Diarrhœa* was the most constant symptom. It was present in ten out of fourteen animals. It made its appearance between the fourteenth and the twenty-fifth day of the experiment, and persisted either as a frank diarrhœa or merged into true dysentery. In six cases (*vide* Table I), the symptoms of diarrhœa persisted up to the time of death ; in four they merged into those of true dysentery. The diarrhœic motions were small, numerous and pale in colour, resembling pea-soup. Microscopical examination showed the stool to consist mainly of (1) epithelial cells in various stages of disintegration, (2) enormous numbers of bacteria, and—in iodine-stained specimens—(3) a small amount of undigested starch. Amœbæ, but without ingested red blood corpuscles, were present in one case. In animals (Group III) receiving butter in addition to the autoclaved rice, another feature was added to the microscopical characters of the stools : large amounts of fatty acid crystals were present. The stools in these cases were paler than usual, and in two instances distinctly frothy, resembling those of sprue, the resemblance being carried

further by the abnormal quantity of fatty acids found on microscopical examination.

*Dysentery.*—Diarrhœa was preliminary to the onset of dysentery in four cases out of ten. After the lapse of a day or two, during which diarrhœic stools were passed, the motions became mucoid and streaked with bright red blood; later they consisted of pladgets of tough blood-stained mucus, sometimes intermingled with traces of pale yellow-white fœcal matter but more often consisting solely of mucus mixed with blood. In two cases dysentery made its appearance without any preliminary diarrhœa. The familiar characters of dysenteric stools were obscured to some extent in these cases by the nature of the animal's food and by the small fœcal output induced by the state of semi-starvation. Nevertheless, they were such as in the human subject would have suggested 'amœbic' dysentery to the mind.

Animals rarely survived the onset of dysentery for more than a few days. In two cases dysenteric stools were passed only for two days prior to the animal's death; in four others they survived the onset of dysenteric symptoms for four, five, six, and seven days respectively.

In the diagnosis of these cases I have had to limit myself to investigation of the microscopical characters of the stool. Bacteriological studies could not, unfortunately, be undertaken. I am, therefore, unaware of the extent to which bacilli may have been responsible for, or participated in, the production of the dysenteric symptoms. There were present in the stools enormous numbers of cellular elements of the most diverse form and size. These elements included, in addition to amœbæ in some cases, (1) desquamated epithelial cells in various stages of disintegration, appearing singly or in connected groups of a dozen or more, (2) red blood corpuscles always in considerable numbers, (3) leucocytes and pus cells in large numbers, (4) single, double, and, more rarely, four-nucleated small round cells, and (5) large mononuclear cells ranging in size up to  $25\mu$  or even more. The majority of these cells showed degenerative changes.

In arriving at a diagnosis in any particular case I have followed the rule laid down by Wenyon and O'Connor<sup>(2)</sup> and called no case 'amœbic' unless I found 'at least some amœbæ with included red blood corpuscles present, or definite *E. histolytica* cysts associated with the amœbæ in the stool.' On this basis, four cases were classed as 'amœbic' dysentery. Amœboid organisms of varying sizes up to  $25\mu$  were present in a fifth case, but as these did not contain ingested blood

corpuscles nor were associated with *E. histolytica* cysts, this case was not classed as 'amoebic.' In the sixth case no amœbæ were found; the histological characters of the stool in this case were such as to render the diagnosis of bacillary dysentery legitimate on these characters alone.

In order to determine whether the monkeys had been amœbæ carriers the fæces of eight healthy control animals were examined. One examination only was made. *E. tetragena* cysts were found after prolonged search in one case, *blastocysts* (considered by Flu<sup>(3)</sup> to be degenerative forms of *E. tetragena*) were found in moderate numbers in a second and in very large numbers in a third; *E. coli* were present in three others. No amœbæ were found in the fæces of the two remaining control animals.

Throughout the greater part of the experiment twelve control monkeys remained free from gastro-intestinal disturbances of any kind. Towards its close, however, seven of them developed jaundice. This was thought to be due to a too generous provision of monkey-nuts with lack of exercise. A meagre diet of plantains and milk, to which was added a pinch of Epsom salts for a few days caused the jaundice to clear up. In no case did diarrhœa or dysentery occur.

It is possible that flies may have conveyed *E. tetragena* cysts or dysenteric bacilli from infected to non-infected animals in the same room. Since, however, the control monkeys did not suffer from dysentery—although equally exposed to any possible infection—it is obvious that the provision of a well-balanced dietary secured their immunity.

This observation indicates (1) that monkeys in the wild state in S. India may be carriers of *E. tetragena* (*histolytica*), and (2) that states of malnutrition favour the multiplication of pathogenic agents of dysentery present in the intestinal tract, whereas a satisfactorily balanced dietary has the reverse effect.

Workers in the Tropics have long been familiar with the fact that cysts of *E. tetragena* (*histolytica*) may be present in the stools of perfectly healthy individuals, and absent from the stools in a proportion of cases possessing the clinical characters of the type of dysentery called 'amoebic' (Manson). I know of no work, however, designed to determine the percentage of *E. tetragena* carriers amongst natives of British India, although doubtless such exists; but recently Flu<sup>(3)</sup> has estimated that at least 10 per cent of natives of the Dutch Indies are *E. tetragena* carriers, and has concluded that this estimate is probably much too low. Wenyon and O'Connor<sup>(2)</sup> also have found 4·5 per cent of *E. histolytica* carriers

amongst healthy British troops in Egypt who gave no history of dysentery. They found also that 13·5 per cent of healthy natives of Egypt were *E. histolytica* carriers. It appears, therefore, that *E. tetragena* (*histolytica*) may exist in the healthy intestine as a 'pathogenic saprophyte' without giving rise to dysentery until the conditions requisite for its growth upon and in the intestinal mucosa are provided by certain favouring circumstances. Amongst these circumstances, malnutrition—including deficiency of accessory food factors—is one. It appears probable also that bacillary dysentery is favoured in its origin by like circumstances.

These observations have a practical bearing in regard to the prevalence, prevention and cure of diarrhoea and dysentery. They provide an explanation of the occurrence of 'famine dysentery' so well recognized in India during times of food scarcity. The specific agents of dysentery, whether recently ingested or existing as 'pathogenic saprophytes,' find in the mal-nourished state of the intestinal mucosa of famine-stricken individuals the conditions necessary for their unhampered growth. It is not necessary to assume a fresh amœbic infection in every case of amœbic dysentery, since the native of India, no less than *macacus sinicus*, may be considered to be often an amœba-carrier.

The frequent occurrence of dysentery amongst the starving inhabitants of certain occupied territories during the late war has probably a like explanation. No doubt both war and famine more often provide greater facilities for the entry of the specific micro-organisms of dysentery into the human intestine than do the more sanitary conditions of peace and plenty; but while recognizing this the present experiment brings into special prominence the importance of suitable dietaries in preventing the onset of this malady. It suggests also that disturbance of gastro-intestinal function from any cause, such, for example, as a malarial attack, may lead to the recrudescence of acute symptoms of dysentery in carriers of the specific agents of this disease or determine a first attack in persons recently infected.

In the prevention of dysentery there are two precautions necessary: (1) the maintenance of the healthy and protective activity of the gastro-intestinal mucosa, and (2) the prevention of infection. I venture to think that the conditions necessary for infection of the intestinal mucosa differ but little from those necessary for infection of the skin; these conditions are provided by ill-nourished, poisoned or necrosing tissues. Improper food-supply is the most ready means of inducing such changes in the gastro-intestinal tract.

The demonstration of the influence of malnutrition in the causation of dysentery may have an important bearing on the prevalence of this malady in jails and asylums. The rice dietaries of Indian jails are, in those cases where I have ascertained their composition, often too close to the borderland of insufficiency with respect to their protein, fat and vitamine content. Thus in one jail in the Madras Presidency, the 'Rice Diet' consists of rice 20 ozs., salt  $\frac{3}{4}$  ozs., tamarind  $\frac{1}{2}$  oz., curry powder  $\frac{1}{4}$  oz., oil (vegetable)  $\frac{1}{2}$  oz., dhal 5 ozs., vegetables 6 ozs. The rice is polished and lacks an adequate supply of accessory food factors of the 'B' class. This deficiency may, to some extent, be compensated for by the five ounces of dhal, provided that the dhal is not deprived of its germ and pericarp. But, in Southern India, so far as I can ascertain, dhal (*arhar*) without its pericarp is chiefly eaten. In Northern India "Dhal Urd" (Bengal gram) and "Mung dal" are used—in these the pericarp is retained. Half an ounce of vegetable oil does not provide a sufficiency of fats or of accessory food factors of the 'A' class, while accessory factors of the 'C' class, contained in the vegetable components of this dietary, are probably largely destroyed in the process of cooking. Such a ration is, therefore, too rich in carbohydrates and too poor in animal fats and proteins, while there is too small a margin of safety with respect to its 'vitaminic' content. It seems probable that 'jail dysentery' may to a great extent be capable of prevention by the more generous and judicious use of appropriate articles of food.

The cessation of this experiment afforded me an opportunity to observe, on the substitution of an adequate dietary, the rapid disappearance of diarrhoea (and of dysentery in one case) in monkeys reduced to a grave state of asthenia by the deficient dietary. In the treatment of dysentery it is necessary to take care that the milk and rice water dietaries so commonly employed are not lacking in vitamins. If they are, not only will recovery from the dysenteric process be greatly impeded but symptoms of beriberi will be prone to manifest themselves. The literature contains many examples of the association of dysentery with beriberi due largely to these causes.

#### PATHOLOGICAL EVIDENCES OF GASTRO-INTESTINAL DISEASE.

The animals were, for the most part, autopsied immediately after death. Where this could not be done the bodies were placed in cold storage and a post-mortem carried out twelve to eighteen hours later. In thirteen cases, the heart's blood was cultured by aerobic methods at

autopsy. Growths were obtained in five cases; with one exception autopsy was performed in these cases immediately after death. The organisms recovered from the blood—usually coliform organisms and small cocci—no doubt indicated the presence of so-called ‘terminal’ infections. It was noted, however, that the adrenalin-content of the supra-renal glands was considerably lower in septicæmic cases than in the majority of animals from whose heart’s blood no organism was grown. In the former the average adrenalin-content was 0·00026 gram, in the latter 0·00039 gram. This circumstance suggests that hæmic infections existed in the former cases some considerable time prior to death and probably hastened it. As will be seen, the diseased state of the gastro-intestinal tube is such as to render hæmic infections extremely likely to occur. Since only aerobic methods of culture were employed it is very probable that undetected hæmic infections existed in other cases; this probability is increased by the low adrenalin-content of the supra-renals in two such cases.\*

#### A. EXTERNAL APPEARANCES OF THE GASTRO-INTESTINAL TRACT.

On opening the abdomen it was observed that the abdominal wall was greatly thinned and completely devoid of fat. The glands of the groin were often enlarged. The *omentum* existed as a thin, transparent membrane from which all traces of fat had disappeared. In healthy monkeys it was found to weigh from fifty to fifty-six grams, while in monkeys dying in consequence of the deficient food it weighed but three to four grams; the loss of weight was due mainly to loss of omental fat. Its vessels were rarely engorged. It closely enveloped the whole mass of intestines being snugly tucked underneath their lower border at the level of the pelvis.

The *mesentery* also was excessively thinned, but its vessels were rarely engorged. The *mesenteric glands*, especially those of the colonic mesentery, were invariably much enlarged. In healthy monkeys these structures can just be distinguished by the naked-eye as small, oval, pink bodies lying at the attachment of the mesentery with the bowel; their size in health did not appear to exceed that of a canary seed, except

\* During the course of my study of the pathogenesis of deficiency disease, I have, at the date of writing (12th July, 1919), estimated the adrenalin-content of the adrenals in 110 animals (pigeons, guinea-pigs and monkeys). I have invariably found a low adrenalin-content in all cases where hæmic infections were demonstrated.—R. McC.







PLATE XXXIII.



Fig. 1.—Photograph of the gastro-intestinal tract of a healthy control monkey. Animal killed twelve hours after last meal. Note normal size of empty stomach, uniform calibre of small bowel, also appearance of healthy colon when partly loaded with faeces. The bulgings in the colon are due to accumulations of faeces. Compare with Fig. 3 (A), showing appearance of colon after bowel has been emptied naturally. Note normal appearance of longitudinal bands of muscle fibres and extent of puckering in the loaded bowel.

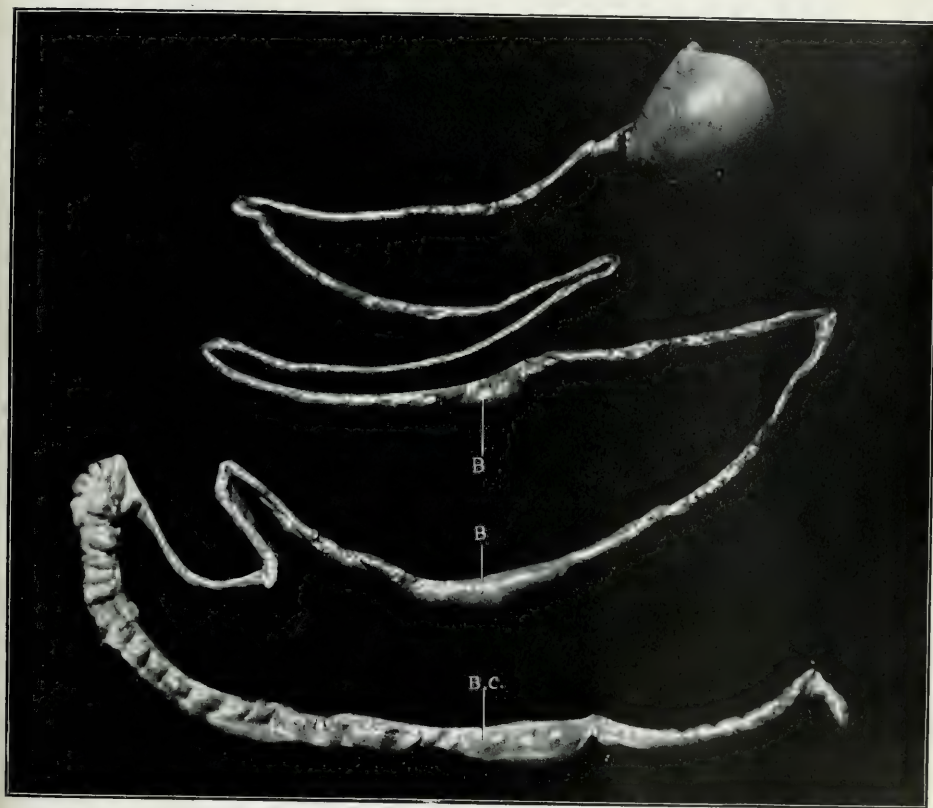


Fig. 2.—Photograph of the gastro-intestinal tract of monkey fed exclusively on autoclaved rice and water. Note great dilatation of the empty stomach, thinning of the small intestine, ballooned areas of small intestine at B, appearances of empty cecum and colon, atrophy of the longitudinal muscular bands of colon, disappearance of puckerings in transverse and descending colon, ballooning of colon at area marked 'B.C.' The ballooning in this case was not due to accumulations of intestinal contents.



internal and posterior to the cæcum where they were sometimes as large as a hemp-seed or even of a small pea. In monkeys dying in consequence of the deficient dietary the mesenteric glands ranged in size from that of a hemp-seed to that of a haricot-bean. Especially in the vicinity of the cæcum they were found to be very prominent. Posterior and internal to this part of the viscus a large mass of greatly enlarged and often discoloured glands was invariably found. In colour they varied from a pale pink to a dull grey or a dark slate-grey colour; in some cases they were of a grey-black tinge. There is no more prominent morbid anatomical feature in these cases than the great enlargement, and the frequent discolouration, of the colonic mesenteric glands; it is an indication of the intense degree of toxic absorption (with which may be included bacterial migration) which takes place from the diseased bowel in these cases. In only one case were the enlarged mesenteric glands examined by cultural methods: a coliform organism, in association with a small coccus, was obtained in this case by aerobic methods of culture.

The *stomach* was invariably greatly dilated and its walls much thinned (Figs. 1 and 2). It but rarely showed external evidences of congestion.

The *small intestine* was always greatly thinned, either throughout its whole extent or more particularly at certain areas. Ballooning of the small bowel was present in eight cases out of fourteen. The ballooning occurred usually in the ileum. The ballooned areas were as a rule multiple and of varying extent; in some cases they were confined to an inch or two of the bowel (Fig. 2), in others much larger areas were involved. In one case (butter-fed), the whole gastro-intestinal tract from the stomach to the rectum was greatly ballooned. The ballooning was not due to the accumulation of intestinal contents at these areas but to excessive thinning of the bowel walls.

*Intussusception* was present in ten cases out of fourteen. The invaginations were usually multiple, invariably descending—the upper portion of the bowel being invaginated into the lower—often of small size, and never showing evidences of inflammation (Fig. 4). They were found at all parts of the small intestine from the jejunum downwards; in one case only was the intussusception ileo-cæcal. The majority of these intussusceptions were no doubt manifestations of the death agony. In some, however, although no inflammatory changes had occurred, considerable constriction was present; the invaginated bowel being then much engorged and sometimes of a reddish brown hue. It is concluded

that such invaginations occurred at least several hours prior to death. I am disinclined to regard them as wholly agonal in origin; agonal intussusceptions are said to be usually ascending, those in the present experiment were invariably descending. It appears probable that the changes in the neuro-muscular mechanism of the bowel, presently to be described, are concerned in their origin.

Congestion of the small intestine was usually a prominent feature; small ecchymoses were frequently found widely distributed under the serous coat. Sometimes these ecchymoses involved the small bowel in its entire length; sometimes they were limited to the duodenum or to the lower end of the ileum or both. As a rule the congestive process was more marked in the duodenum than in other parts of the small intestine. A notable feature in monkeys fed on autoclaved rice *plus butter* was the pale yellow-white colour of the entire gastro-intestinal tube.

On opening the abdomen, the external evidences of disease presented by the *large intestine* were the following: (1) congestion and subperitoneal ecchymoses, (2) ballooning, (3) thinning and partial disappearance in places of the longitudinal bands of muscle.

The congestive process affected the great bowel in the same manner as the small. Sometimes subserous ecchymoses occurred throughout its entire length from the tip of the cæcum to the lower end of the rectum. More usually the ecchymoses were limited to certain areas, frequently to its last four to six inches. Occasionally grey-black lymphoid nodules of the size of a small pea projected into the serous coat. The colons of butter-fed monkeys were usually of a pale yellow-white tint in marked contrast to the pink tinge of health.

The ballooning of the colon affected in rare cases the whole organ from the cæcum downwards. As a rule, however, it was limited to certain areas (Figs. 1, 2 and 3). At these areas the characteristic puckerings of the great bowel, produced by the three longitudinal bands of muscular fibres, were wanting or much less prominent than in health. The bowel walls were greatly thinned, the longitudinal bands of muscle being less evident than in health: sometimes the naked-eye could only detect with difficulty the longitudinal bands at these areas; then they seemed to terminate abruptly at the upper extremity of the ballooned area and to commence again at its lower. When placed in formalin, however, the thinned bands could be clearly seen. As a rule the ballooned areas involved the whole circumference of the bowel, but occasionally they



Fig. 3.—Colon: (A) of normal monkey. (B) of monkey fed on autoclaved rice, (C) of monkey fed on autoclaved rice plus butter.





were limited to one side of the bowel resembling then a weak spot in an inflated inner cycle tube. They were not due to faecal accumulations. Between these ballooned areas the colon often presented a comparatively normal appearance with respect to its longitudinal bands and puckerings (Fig. 3). Details with regard to these ballooned areas in several cases will be found in the Appendix.

The external appearances of the gastro-intestinal tract thus presented evidences of grave derangement of bowel function.

#### B. INTERNAL APPEARANCES OF THE GASTRO-INTESTINAL TRACT.

The *stomach* was always greatly distended and quite empty (Figs. 1 and 2). The hand-lens showed its mucous membrane to be abnormally soft and necrotic. Ecchymoses were frequently present; usually at the pyloric end of the viscus. They varied in extent and in degree; in one case (No. 29 Appendix), the whole mucous membrane of the stomach, with the exception of a small portion of the fundus, was covered with minute ecchymoses. In thirteen cases no ulcers were present; in the fourteenth (No. 30) an ulcer of the size of a three-penny bit was present at the pyloric area.

The *duodenum*: congestive and necrotic changes in the mucous membrane were the chief features observed in this part of the bowel; these changes were often very marked. No ulcers were met with. As a rule the ecchymoses of the mucous membrane were much more pronounced in the duodenum than in the stomach and were sometimes seen to commence sharply, or more properly to be sharply accentuated, at the distal side of the pylorus. The whole surface of the duodenum was often studded with small pin-point hæmorrhages, the mucous membrane appearing as if it had been dusted with pepper. The upper part of the duodenum was, as a rule, more severely affected than the lower.

The congestion and ecchymoses frequently extending to the *jejunum* and *ileum* and in exceptional cases this 'peppered' appearance of the bowel was continued to the ileo-cæcal junction. More usually, however, the congestion and ecchymoses were confined to the upper part of the small intestine or to the lower ileum, the intervening areas being comparatively free except where intussusceptions had occurred. In general the invaginated portions of the bowel were more congested than other parts of the small intestine—the duodenum excepted—the congestion

in some intussusceptions being pronounced. Above areas where the more extensive invagination of the bowel had occurred its lumen was often filled with a mucoid material resembling in appearance thin glue; this material was only present above the larger, and apparently older, intussusceptions where it was sometimes blood-stained. Where ballooning had occurred the walls of the bowel were often so thin as to be transparent, and here also a thin glue-like mucoid material was sometimes present in the bowel lumen. Often, however, these areas were empty of all contents except gas.

As a rule the naked-eye changes were more pronounced in the *large intestine* than at any other part of the gastro-intestinal tract—the duodenum excepted. The changes were those of an intense colitis but without ulceration. Although amœbic dysentery was present in a number of cases dying in consequence of the deficient dietary, no localized ulceration had occurred in any of them. The colitis sometimes involved the whole extent of the colon, but more usually it was confined to the last six inches of the great bowel; it usually corresponded in distribution to that of the subserous ecchymoses. In one case it assumed its maximum proportion at an isolated area of the mid-colon. The mucous membrane of the cæcum was usually moderately congested and ecchymotic and often of a dark slate-grey colour. The lymphoid nodules of the colon were sometimes very prominent: in two cases they formed dark masses, the size of a small pea, which projected into the lumen of the bowel and into its serous coat; one of these nodules showed on section numerous collections of small cocci. At areas where ballooning had occurred the walls of the colon were excessively thin, often almost transparent. On opening the bowel at these points the walls collapsed like a burst balloon.

\* It is of interest to record the fact that amongst sixteen wild monkeys in which the gastro-intestinal tract was minutely studied at autopsy neither nematodes nor other intestinal parasites were found.

In connexion with the pathological processes initiated in the gastro-intestinal tract of monkeys by an autoclaved rice dietary, the post-mortem appearances found in two cases of berberi by Willcox<sup>(2)</sup> during the Dardanelles campaign may be quoted: 'The stomach showed marked redness of the mucous membrane, which was most marked in the pyloric half where the colour was deep crimson. The duodenum showed intense crimson congestion of the mucosa, most marked in the upper part. The jejunum and ileum showed marked congestion, some petechiæ being

present in the ileum. The large intestine showed congestion. Numerous small hæmorrhagic patches about half-an-inch in diameter were present in the wall of the ascending colon. The mesenteric glands showed slight enlargement.' The similarity of these changes to those found in monkeys fed on autoclaved rice is very noteworthy.

It is noteworthy also that changes in the duodenum and small intestine similar to those I have described, but of lesser severity, can be produced in rabbits by the oral administration of 5-10 c.c. of ox-bile. The lesions so produced admit of the implantation of the typhoid bacillus on the intestines of rabbits if the dose of ox-bile is followed some hours later by the oral or intravenous administration of a typhoid culture. Without the preliminary dose of ox-bile the ingestion of the typhoid organisms is harmless<sup>(6)</sup>. It seems probable, therefore, that immunity to gastro-intestinal infections is largely a question of the integrity and health of the gastro-intestinal mucosa.

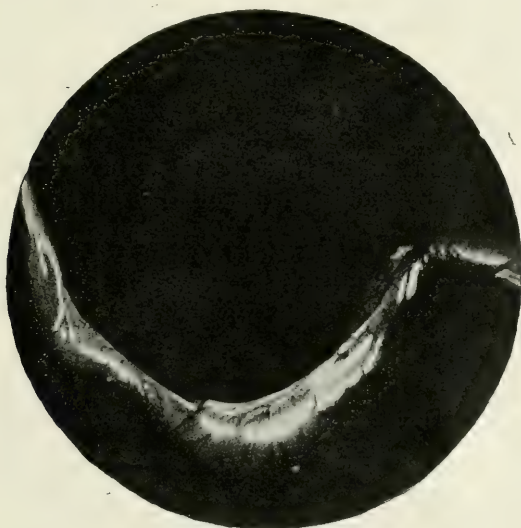


FIG. 4.—Photograph of descending ileal intussusception in monkey fed exclusively on autoclaved rice.

## C. HISTO-PATHOLOGY.

The histo-pathological changes were studied in all fourteen cases and were contrasted with the normal appearances in healthy monkeys.

In order to avoid post-mortem changes in the tissues, portions of the cardiac and pyloric ends of the stomach, of the duodenum, of the jejunum, of the lower ileum, of the ballooned areas of the small intestine, of the colon and of the ballooned areas of colon were taken for histological study in each case as soon after death as possible. The tissues were fixed in Zenker's fluid and stained by Mann's stain, Unna's methylene blue and eosin, and Heidenhain's iron-haematoxylin.

The histo-pathological changes comprised—

- (1) congestion and hæmorrhage,
- (2) atrophy of the myenteron,
- (3) degenerative changes in the myenteric plexus of Auerbach,
- (4) atrophic, necrotic and inflammatory changes in the mucous membrane,
- (5) bacterial invasion of the bowel walls, and
- (6) partial disappearance of lymphoid elements of the mucous membrane.

*Congestion and hæmorrhage.* Where congestion occurred it was confined to the mucous, submucous and serous coats of the stomach and bowel. The myenteron was not involved or but slightly. In this respect the appearances presented by sections were in marked contrast to those previously described in the case of pigeons and guinea-pigs<sup>(5)</sup>. Small collections of effused blood corpuscles were frequently encountered under the serous covering of the bowel—usually in sections from the duodenum and colon. It is to be noted that congestion did not occur uniformly throughout the entire digestive tube; it was less marked in the mid-portion of the small intestine. The vessels of the duodenal and colonic submucosa were engorged and actual hæmorrhages around the bases of the glands of Lieberkühn were not uncommon (Fig. 8). Free blood corpuscles were often met with lying amongst the necrotic cells of the mucous membrane in sections from the stomach, duodenum and colon. The photo-micrographs illustrating the appearances seen in the diseased mucous membrane of the stomach (Figs. 5 and 6), sufficiently exemplify this point.

*Atrophy of the myenteron.* This atrophy varied in degree at different parts of the bowel. It was extreme in areas where

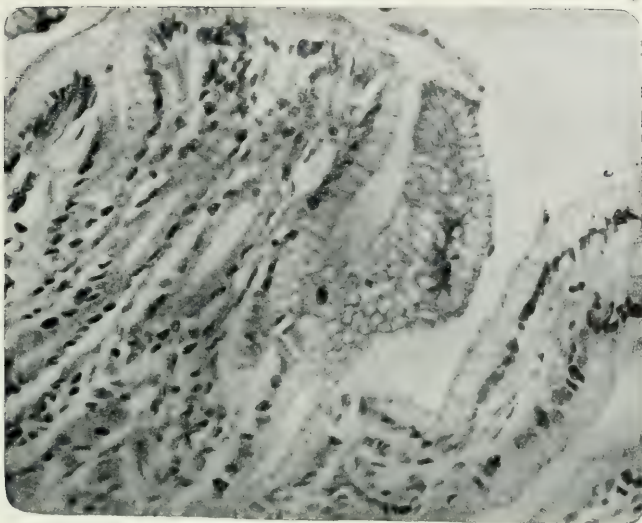
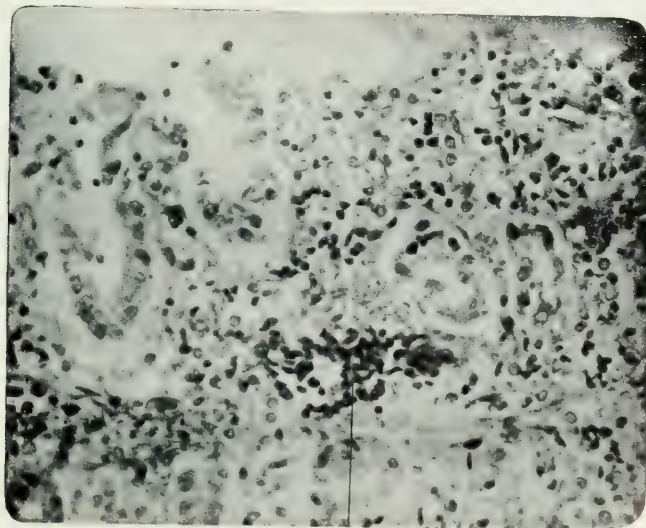


Fig. 5.—The Stomach : pyloric end. Normal Monkey.



H.

Fig. 6.—The Stomach : pyloric end. Diseased Monkey.





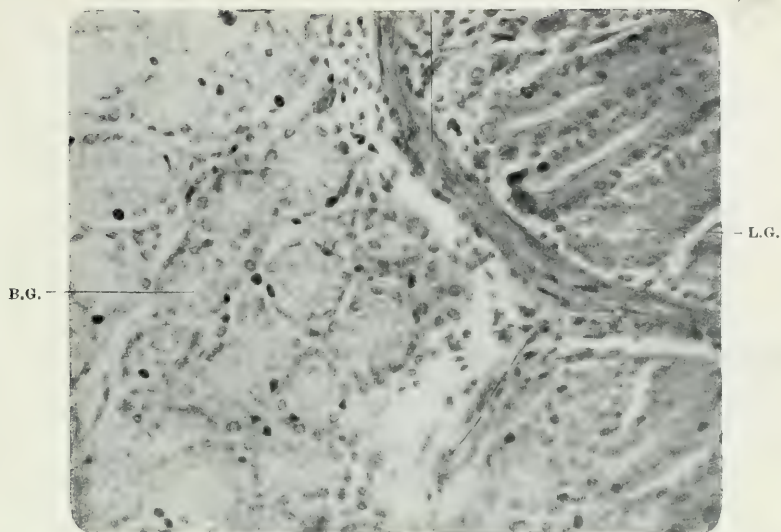


Fig. 7.—The Duodenum. Normal Monkey.

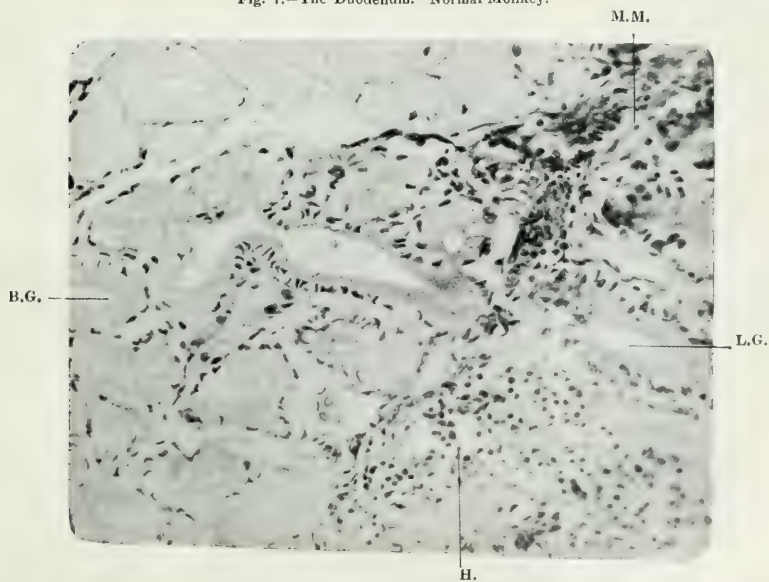


Fig. 8.—The Duodenum. Diseased Monkey.





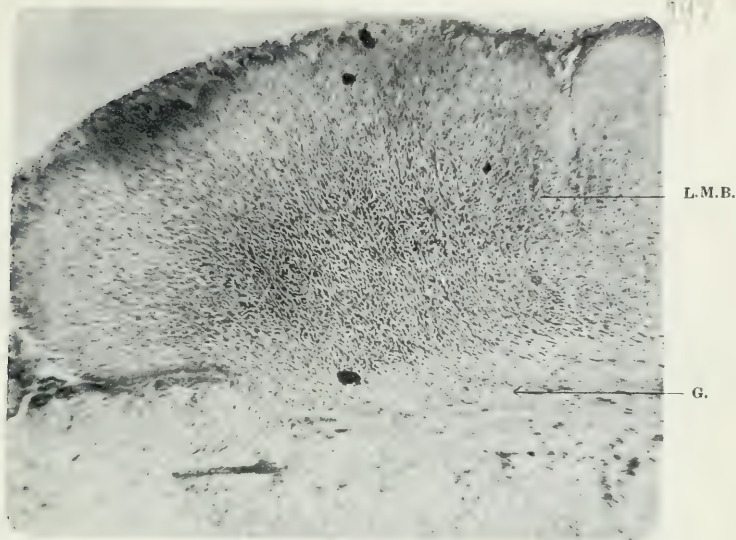


Fig. 9.—The Colon. Normal Monkey.  
H.

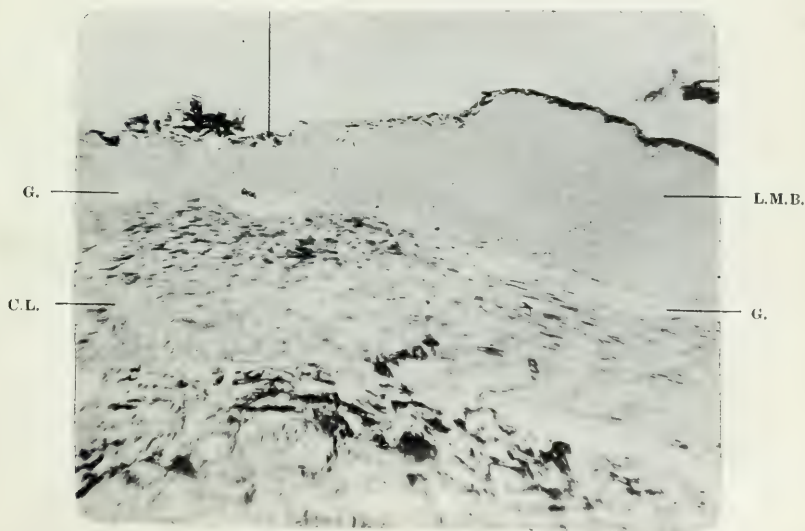


Fig. 10.—The Colon. Diseased Monkey.



PLATE XXXIX.

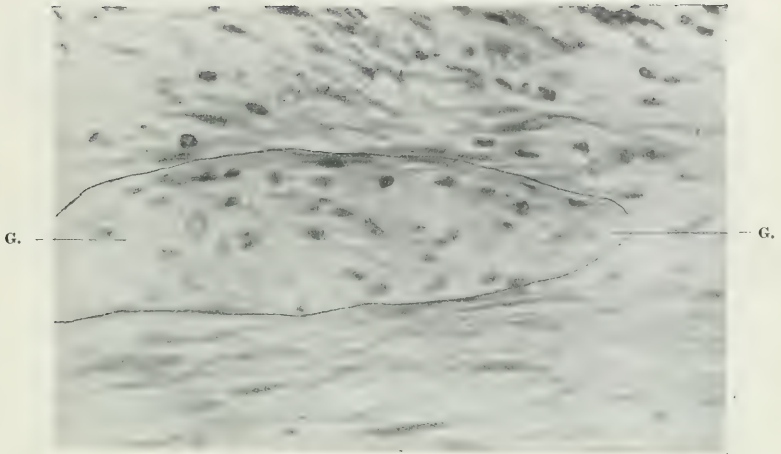


Fig. 11.—Ganglion of Auerbach. Normal Monkey.

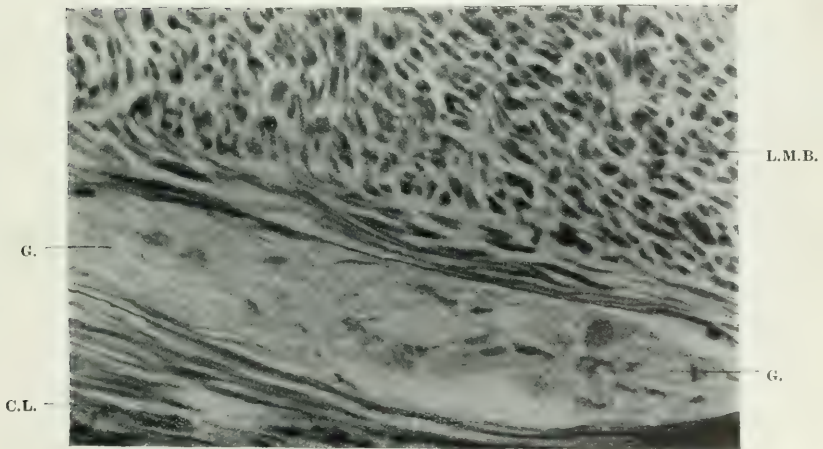


Fig. 12.—Ganglion of Auerbach. Diseased Monkey.



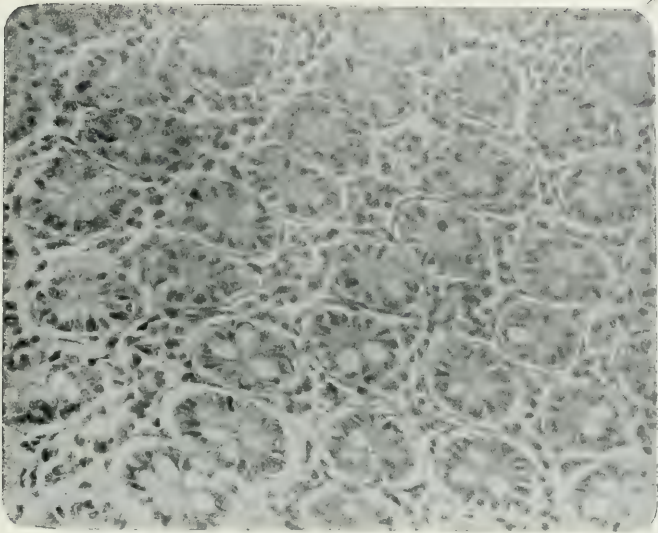


Fig. 13.—The Mucous Glands of Colon. Normal Monkey.

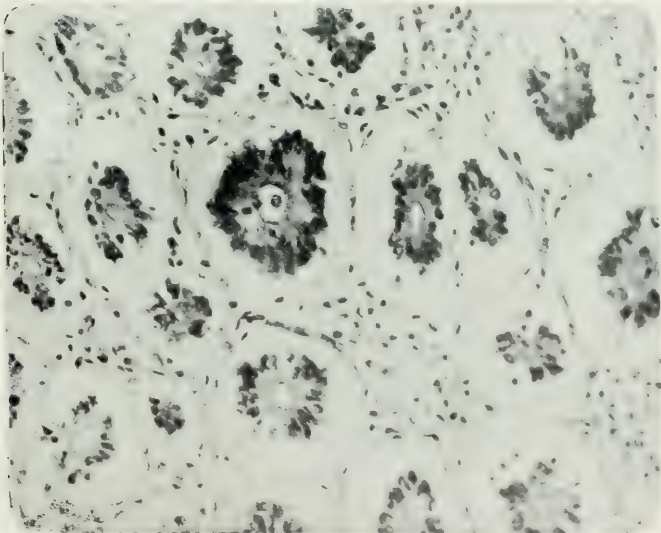


Fig. 14.—The Mucous Glands of Colon. Diseased Monkey.





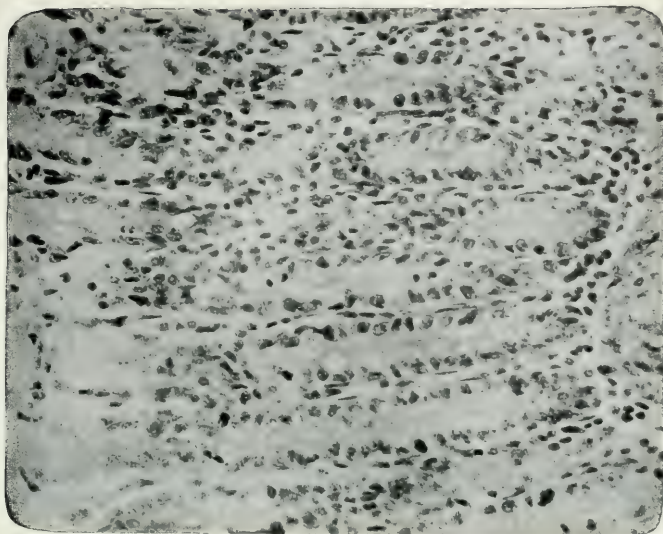


Fig. 15.—The Superficial Glands of Colon. Normal Monkey.

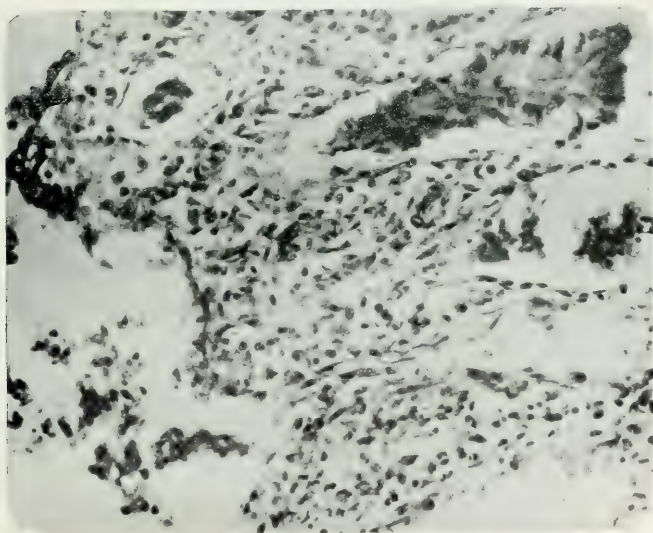


Fig. 16.—The Superficial Glands of Colon. Diseased Monkey



ballooning had occurred. The dilatation of the stomach was probably less a matter of atrophy of its muscular coats than of their inability to contract. Apart from the ballooned areas of the bowel, where the myenteron presented extreme degrees of tenuity, the most remarkable changes were found in the longitudinal bands of the colon. Figs. 9 and 10 represent the degree of thinning of these bands. These sections were taken from approximately the same part of the colon in the healthy as in the diseased state; the magnification is the same in both. It will be noted that the thinning of the longitudinal bands is due in part to their spread over the surface of the distended bowel; in part also it is due to atrophy of muscle fibres.

*Degenerative changes in the myenteric plexus of Auerbach.* In all parts of the gastro-intestinal tract the ganglia of the myenteric plexus often presented appearances in marked contrast to those of health. Although the stains employed were not such as to bring out in minute detail the degenerative changes in individual cells, nevertheless they were sufficient to indicate the existence of such changes. The ganglia were much more prominent in the diseased bowel. In Figs. 9 and 10 the contrast in this regard between health and disease is well illustrated; in the healthy intestine (Fig. 9) the chain of ganglia lying between the longitudinal band and the circular layer of muscle can be seen with difficulty, whereas in the diseased bowel (Fig. 10) it is a prominent feature. The ganglionic cells were swollen, the cell membrane indistinct, frayed or wanting. The nuclei had often disappeared or existed only as fragments of chromatin; where complete nuclei were found they were often swollen. The nucleolus was frequently fragmented. Occasionally lacunæ empty of cells were found in the degenerating ganglia (Figs. 11 and 12). Ganglia were sometimes encountered in which no cell contained a normal nucleus; others in which a few cells appeared relatively normal, and others in which degenerative changes were slight or absent. There can be no doubt that such changes would impair the neuro-muscular control of the bowel.

*Necrotic and inflammatory changes in the mucous membrane.* These consisted in necrosis of all cellular elements of the mucous membrane. The necrosing cells were often surrounded by inflammatory cells and effused blood corpuscles. The duodenum (Figs. 7 and 8) and colon (Figs. 13 to 16) were in general most severely affected in this regard; some degree of colitis was present in the great majority of cases. The mucous membrane of the stomach often exhibited necrotic and congestive

changes (Figs. 5 and 6). In ballooned areas of both small and large bowel, only remnants of mucous membrane were met with. All glandular elements of the gastro-intestinal tract were affected in greater or lesser degree—the gastric and pyloric glands (Figs. 5 and 6), the glands of Brunner in the duodenum (Figs. 7 and 8), the mucous glands of the colon (Figs. 13 and 14), and the glands of Lieberkühn (Figs. 7 and 8). Throughout the entire intestinal tract the muscularis mucosæ was greatly thinned (Figs. 7 and 8), the atrophy in places constituting actual breaches in its continuity. The photo-micrographs illustrate the appearances seen so clearly that no further description of them is called for.

Considerable variation in the severity of the lesions in different animals and in different parts of the same gastro-intestinal tract was noted.

*Bacterial invasion of the bowel walls* was encountered in a number of suitably stained specimens, especially from the colon. Bacteria in large numbers were often seen deep in the mucous membrane; sometimes they formed a bacterial capsule around the desquamated cells of the glands, as, for example, around those of the mucous glands of the colon. Frequently they were seen scattered throughout the sul. mucosa, and mycelium-like organisms were, in several specimens, found lying in small cavities in this coat of the bowel. In some sections from the colon the bacterial invasion was seen to extend to the muscular layers, and bacteria in considerable numbers were found lying in and between the layers of the myenteron. More rarely organisms were found in the serous coat or in blood-vessels. Large collections of cocci were found in an enlarged lymphatic nodule taken from the colon. The organisms, as seen in sections of the colon, were of four varieties: (1) cocci, (2) short rods, resembling *B. coli*, (3) longer rods, usually found in and between the muscular coats, and (4) mycelium-like organisms and their spores, usually found in small cavities in the submucosa.

*Atrophy of lymphoid elements.* Although in the colon the lymphoid nodules were frequently swollen, in general the lymphoid elements of the intestinal mucous membrane were greatly reduced in numbers. In health the lymphoid cells are very abundant, whether collected into nodules or filling in the spaces between the glands of Lieberkühn. The thinning of the protective layer of lymphocytes no doubt facilitated the development of infective processes in the mucous membrane.

Clearly all these changes are such as would favour greatly hæmic infection from the diseased bowel.

## SUMMARY OF RESULTS.

The effects of autoclaved rice dietaries on the gastro-intestinal tract of monkeys may be summarized as follows :—

(1) Congestive, necrotic and inflammatory changes in the mucous membrane of the entire tract.

(2) Degenerative changes in the neuro-muscular mechanism of the entire tract, leading to dilatation of the stomach, ballooning of areas of the small and large bowel, and probably also to the intussusceptions which so frequently occur.

(3) Pronounced necrotic changes in the secretory elements of the entire gastro-intestinal tract—of the gastric glands, the pyloric glands, the glands of Brunner, the glands of Lieberkühn, and of the mucous glands of the colon. These changes are such as must cause grave derangement of the digestive and assimilative processes.

(4) Intense toxic absorption from the diseased bowel as evidenced by the changes in the colonic mesenteric glands.

(5) Impairment of the protective resources of the entire gastro-intestinal mucosa against infecting agents, leading to infection of the mucous membrane by 'pathogenic saprophytes' and by ingested pathogenic organisms. These infections give rise to gastritis, enteritis, and to colitis and frequently to hæmic infections. The occurrence of dysentery in these circumstances is a most significant finding.

These results are very similar to those I have described in the case of pigeons and guinea-pigs fed on ill-balanced dietaries deficient in accessory food factors<sup>(5)</sup>.

## COMMENTARY.

Such degrees of malnutrition as those brought about under the conditions of the present experiment are rarely encountered during peaceful phases of our civilization. But the late war afforded many examples of gastro-intestinal disorder (amongst refugees, prisoners of war in Germany and Turkey, and the starved inhabitants of certain occupied countries) which find their pathological explanation in the facts here recorded. These facts represent an extreme picture: the end results of malnutrition. But between this extreme on the one hand and perfect nutrition on the other, there are intervening degrees of malnutrition which must determine the departure from health of the gastro-intestinal tract. It is with the 'mean' rather than with the 'extreme' that the physician is mainly concerned; with the long continued use of

imperfectly balanced and deficient food rather than with the lack of victuals; with the beginnings of disease rather than with its end results.

In the present experiment the gastro-intestinal lesions I have described were initiated by dietaries too rich in carbohydrates and too poor in other essential attributes of a perfectly balanced ration. With this combination the physician is familiar in the dietaries of human beings. It may be expected then that gastro-intestinal lesions of like kind, if not of like degree, will result in man from the continued use of such dietaries.

To maintain the gastro-intestinal tract in health, and to enable it to protect itself and the body generally against invasion by ingested pathogenic organisms, it is necessary to provide from birth onwards a satisfactorily balanced dietary containing a sufficiency not only of proteins, calories and salts but also of accessory food factors of all three classes.

#### APPENDIX.

##### CLINICAL AND POST-MORTEM RECORDS.

*Monkey No. 1.*—Diarrhoea appeared on 16th day of the experiment. Vomiting on the 17th. Stools became mucoid and blood-stained on 17th day; consisted solely of blood-stained mucus on 18th. Anaemia present; no ingested red blood corpuscles, probably not *E. histolytica*. Died morning of 19th. Post-mortemed immediately.

Abdominal wall devoid of fat as also the omentum. *Omentum* weighs 3.5 grams; very thin, transparent. *Mesenteric glands* much enlarged and of slate-grey colour. Congestion of entire intestinal tract. *Colon*: studded with ecchymoses under serous coat throughout entire length. *Stomach* greatly dilated, filled with air; no food. *Small intestine*: very thin; whole of serous surface studded with small ecchymoses, gradually merging to a reddish brown colour towards invaginated portions of the bowel. At a point six inches above the ileo-caecal valve, there is a descending *intussusception*, forming a sausage-shaped tumour  $2\frac{1}{2}$  long: no inflammatory changes, but invaginated bowel greatly congested and of reddish-brown hue. Bowel immediately above the intussusception is very thin. Contents of bowel at this point consist of mucoid material resembling thin glue which is blood-stained. Five inches above the first intussusception, a second is met with also descending, and one inch long. Invaginated bowel much congested and dark in colour. Four inches above the second intussusception, a third is encountered, also descending: here also the bowel is much congested. Three inches above the third intussusception, a fourth is present. It is of small size ( $\frac{1}{2}$  inch) and the invaginated bowel shows no greater degree of congestion than other non-invaginated portions. Of these four intussusceptions the most marked is that nearest the ileo-caecal valve.

On opening the *stomach* the mucous membrane is seen to be soft and necrotic-looking: many ecchymoses in vicinity of the pylorus. This congestion is greatly accentuated as the pylorus merges into the *duodenum*: the first one-and-a-half inches of this part of the bowel is very congested and its mucous membrane studded with ecchymoses. The congestion and ecchymoses extend in diminishing degree down the *duodenum* and into the *jejunum* and *ileum* becoming again very marked at areas where intussusceptions have



occurred. At these areas (in the case of the three lower intussusceptions) the mucous membrane is very dark and greatly congested. The whole small intestine is empty with the exception of collection of mucoid material above the three lower intussusceptions. The small bowel walls are so thin in parts as to be almost transparent. This thinning is especially marked just above the ileo-cæcal junction.

The mucous membrane of the *colon* shows evidence of severe colitis: intense congestion, ecchymoses, and necrotic changes. These appearances are more pronounced in the last six inches of the colon; no ulceration is present. The mucous membrane of the cæcum is slate-grey in colour.

*Monkey No. 2.*—Diarrhœa appeared on the 21st day, became dysenteric on 22nd day. Stools consisting of blood and mucus persisted until the 26th day when the animal died. Numerous vegetative forms of *E. histolytica* with ingested blood corpuscles present. Animal post-mortemed immediately after death.

The appearances resembled closely those detailed in Monkey No. 1 with the following differences: ecchymoses confined to serous coat of lower half of large bowel and to upper half of the small bowel, the duodenum being most affected. Lower half of small bowel not notably congested but its walls are excessively thin and greatly ballooned in places, especially at a point eight inches above the ileo-cæcal junction. Congestion of the mucous membrane of the duodenum and upper part of the small intestine is very marked. Colitis in this case is even more severe than in Monkey No. 1; most pronounced in its lower half: no ulceration. No intussusceptions.

*Monkey No. 3.*—Diarrhœa appeared on 18th day: no amœbæ; persisted as such until 22nd day when animal died. Vomiting, 19th and 20th days. Post-mortemed 12 hours after death: kept in cold store during this time.

Omentum very thin; great enlargement of the mesenteric glands which are dark slate-grey in colour, very marked at cæcal region, here glands size of haricot-bean. Feature of this case is the ballooning of the *colon*: ballooning occurs at certain points only, intervening areas retaining much of the normal puckered appearance. Ballooning may extend around the whole circumference of the bowel, or involve only one side between two longitudinal bands of muscle or between one such band and the attachment of the mesentery. Of these ballooned areas the first and lowest was situated 2" above the termination of large bowel: it was 2½" in extent and involved the whole circumference of the bowel. Above this area came one inch of fairly normal looking bowel so far as puckering was concerned. This area was succeeded higher up the bowel by a ballooned area involving the whole bowel circumference: it was ½" long and 1" broad. This area was succeeded by 1½" of colon of comparatively normal thickness and puckering. Above this area was unilateral ballooning of the bowel wall involving ½" of its circumference. Above this point the bowel was irregularly ballooned up to the cæcum. At these ballooned areas the bowel walls are excessively thin, almost transparent in some places and actually transparent in others. The longitudinal bands of muscle are greatly thinned over the ballooned areas and spread out or laterally stretched over this surface: these bands are so thin as to be difficult to distinguish in places by the naked-eye.

The *small bowel* is also excessively thin and is ballooned in places. Throughout the whole length of the tract from pylorus to anus the vessels surrounding the bowel are engorged, not more so at one part than at another. Only the lower five inches of colon above the anus show ecchymoses under the serous coat. At this area they are extensive and situated chiefly at the side of the bowel opposite the mesenteric attachment.

On opening the tract the greatly dilated *stomach* shows only a small area of congestion of the mucous membrane close to the pylorus. It is quite empty of food, the mucous membrane being necrotic-looking. The congestion of the pyloric end of the stomach is continued into the *duodenum* but greatly intensified, for six inches along the course of the bowel, when it gradually fades. The ecchymoses give to the mucous



membrane the appearance of having been sprinkled with pepper. The mucous membrane has lost its rugose appearance throughout the whole small intestine. The small intestine is empty except for a sero-mucoid fluid. The *colon* shows colitis especially of lower half. Mucous membrane of *cæcum* dark slate-grey in colour. The bowel is practically empty; the ballooned appearance seems to be maintained by the presence of gas since when cut into, in order to remove part for sectioning, these areas collapse like a burst balloon. Figs. 10 and 12 are from this case.

*Monkey No. 4.*—This animal died suddenly on the 22nd day. It had no diarrhoea or dysentery but vomited occasionally during the last two or three days of life. Post-mortemed immediately.

General appearances as in previous cases. Detail as follows: *stomach*: greatly dilated, shows congestion and ecchymoses of mucous membrane. Pyloric area chiefly involved, no ulcers. No ecchymoses are present on serous surface of bowel. *Ileum* ballooned in places. Three intussusceptions present in small intestine; no inflammatory change. Colitis less marked in this case than in previous cases, it tends to be localized to a limited area six inches below the *cæcum* where the congestion of the mucous membrane is intense. *Duodenum* and upper intestine very congested, especially upper four to five inches where ecchymoses of mucous membrane give appearance of bowel being sprinkled with pepper. Collection of glairy mucoid material occur at ballooned areas of small bowel; at some of these areas the bowel walls are transparent.

*Monkey No. 5.*—Diarrhoea appeared on the 17th day, became dysenteric on the 19th day and persisted to 24th day when animal died. Numerous *E. histolytica* present. Post-mortemed immediately.

General appearances as in previous cases. Detail as follows: greatly dilated *stomach* shows only slight congestion close to pylorus. Large ileo-cæcal intussusception; no inflammation but considerable congestion. Another intussusception  $1\frac{1}{2}$ ' in length six inches below the pylorus. Small intestine greatly thinned; ballooned in places. Intense colitis is present but no ulceration. Considerable ballooning of the *colon*; walls very thin at these areas.

*Monkey No. 6.*—Diarrhoea appeared on the 15th day, became dysenteric on the 17th day; stools contain numerous *E. histolytica*; animal died on 19th day. Post-mortemed immediately.

General appearances as in previous cases. Detail as follows: *stomach* dilated, ecchymoses on mucous surface at pyloric end. Moderate congestion of small intestine which is greatly thinned, especially at lower ileum, ballooned in places. Three small descending ileal intussusceptions; no inflammatory changes. *Colon*: slight ecchymoses chiefly at lower end. Pronounced colitis of lower part of colon. No ulceration.

*Monkey No. 7.* Diarrhoea appeared on 23rd day; vegetative amoebæ present; no ingested red blood corpuscles, not *E. histolytica*. Animal died on 26th day. No dysentery. Post-mortemed 18 hours after death.

General appearances as in previous cases. Detail as follows: the enlarged mesenteric glands are almost black in colour, great collections in neighbourhood of *cæcum*. *Duodenum* much congested. *Small bowel* very thinned; marked ballooning at two points. *Colon* shows moderate colitis and considerable ballooning, especially marked in middle eight inches. *Stomach* is greatly dilated; pyloric end congested. No intussusceptions. The whole gastro-intestinal tract is shown in Fig. 2.

*Monkey No. 8.*—Dysenteric symptoms appeared on the 19th day, without preliminary diarrhoea. No amoebæ were found in the mucous blood-stained stool. Animal died on 26th day. Post-mortemed immediately.

General appearances as in previous cases. Detail as follows: no intussusceptions; *stomach* greatly dilated, no congestion; no ulcers. *Duodenum*: very marked congestion.

*Small intestine*: generalized congestion, ecchymoses of mucous membrane, several areas of small intestine are ballooned, closely resembles previous case (Fig. 2). *Colon*: last four inches of normal calibre, there are many ecchymoses under serous coat at this part. Above it the bowel is greatly ballooned (Fig. 3 B.), the walls are so thin at this point as to be almost transparent. This ballooned area is succeeded by an area of comparatively normal calibre and this again by an extensive but more moderately ballooned area. The cæcum is very wide. The whole colon is shorter than usual. The longitudinal bands of muscle are very faint over lower ballooned area and much thinned throughout whole extent of colon. A very severe colitis is present in lower four inches of colon: the congestion of the mucous membrane stops abruptly at the lower end of the ballooned area. Throughout this area the walls of the bowel are very thin and the mucous membrane very atrophic: no congestion is visible to the naked-eye but a few pin-point ecchymoses can be seen with the aid of the lens. Above this ballooned area the colitis starts again. Three large, black lymphoid nodules project into the lumen of the colon and into its serous coat. The mucous membrane of the cæcum is dark slate-grey in colour. The mesenteric glands are much enlarged and dark slate-grey in colour, especially in the neighbourhood of cæcum.

*Monkey No. 11.*—This animal died suddenly on the 22nd day without showing any gastro-intestinal symptoms. Post-mortemed immediately.

General appearances as in previous cases. Details as follows: mesenteric glands greatly enlarged and dark in colour. A small descending *intussusception* was present 3" above the cæcum, another large double *intussusception* 6" above the first and three other smaller ones up to the jejunum. Mucous membrane of cæcum very dark in colour, generalized congestion of whole tract. Colitis not marked.

*Monkey No. 12.*—Diarrhœa appeared on the 25th day and persisted as such to death of animal on 28th day. Vomiting noted on 26th day. General appearances as in previous cases. Features calling for special notice are excessive dilatation of stomach and moderate-sized ileal *intussusceptions* (Fig. 4).

*Monkey No. 27.*—Diarrhœa appeared on the 12th day and persisted as such until the animal's death on the 15th day. Post-mortemed immediately. The most noteworthy feature of this case is the great thinning of the walls of the gastro-intestinal tract and their pale yellow-white colour. The *stomach* is greatly dilated: there are no ulcers: its walls are very thin and there is considerable congestion and ecchymoses in the pyloric region. Three *intussusceptions* are present in the small intestine: the first six inches from the pylorus, the second 22" from the pylorus, the third 30" from the pylorus. The *intussusceptions* are all descending and have the characters described in previous cases. The small bowel from the pylorus to the ileo-cæcal junction measures  $49\frac{1}{2}$ ". There are five ballooned areas of the small bowel where the walls are practically transparent. The mucous membrane of the duodenum and upper jejunum shows the usual ecchymotic appearances. The *colon* measures from the tip of the cæcum to the neck of the bladder  $20\frac{1}{2}$ ". There are two areas of marked ballooning: at one spot the bowel wall is transparent. At these areas great thinning of the longitudinal muscular bands occur. The colon shows well-marked colitis.

*Monkey No. 29.*—Animal developed dysentery on the 9th day of the experiment. Numerous *E. histolytica* present. Vomiting on 10th and 11th days. Died on the 13th day. No *E. tetrajena* cysts seen. Post-mortemed immediately. *Omentum* very thin: weighs 4 grams. Mesenteric glands very enlarged and dark. Glands cultured: coliform organism in association with a small coccus grown. Great mass of these glands behind and internal to the cæcum. Lower half of colon is enormously ballooned where walls are very thin: its mucous membrane is necrotic and ecchymotic throughout its entire length: severe colitis: no ulcers: mucous membrane of cæcum slate-grey in colour. Several large dark lymphoid nodules project into mucous and serous coats of the bowel. In the small bowel the chief features are the pallor, and great thinning and ballooning

of the bowel walls; its mucous membrane is not markedly congested or ecchymotic. The stomach is greatly dilated and its whole mucous surface with the exception of a small part of the fundus is intensely ecchymotic. This congestion extends into the duodenum for a short distance.

*Monkey No. 30.*—Diarrhœa appeared on the 10th day and persisted until death of the animal on the 14th day. Motions frothy, contain many fatty acid crystals. Post-mortemed immediately after death. General characters as in previous cases. Detail as follows: *small intestine* excessively thin, distended and pale in colour. Two *intussusceptions* are present, both descending, the tumour being one inch in length in both. The first is situated 11" from the pylorus, the second 30". Length of small intestine from pylorus to ileo-cæcal junction is  $51\frac{1}{2}$ ". Congestion is marked at areas of invaginated bowel, in duodenum and in lower 9" of the ileum. The stomach is greatly dilated: an ulcer, the size of a three-penny piece, is present at the pylorus: considerable ecchymosis is present at pyloric end of the stomach. Mucous membrane of the *duodenum* is greatly congested and ecchymotic: congestion extends to mucous membrane of jejunum; it is not marked or is absent until the lower end of the ileum is reached when it is again pronounced. *Colon* is 24" long. No ecchymoses are present in its serous coat. Internally the mucous membrane is necrotic with scattered ecchymoses occurring at the lower part of the bowel. Throughout the whole extent of the colon the lymphoid follicles are greatly swollen. When the thin bowel is held up to the light, these structures stand out clearly studded all over it.

*Monkey No. 36.*—Sprue-like diarrhœa appeared on the 10th day of the experiment: the frothy stools, containing many fatty acid crystals, persisted until the 18th day when the animal died. Post-mortemed immediately after death. Feature of this case is the enormous dilatation of the whole gastro-intestinal tract and its pale yellow-white colour. There is a small *intussusception* 6" from the pylorus. Mesenteric glands much enlarged: they are of a dark greyish colour; the glands in the vicinity of the cæcum are very much enlarged and discoloured. The whole small bowel is extremely dilated and the walls very thin. The small bowel measures  $42\frac{1}{2}$ "; the dilatation appears to have reduced its length. The cæcum is greatly distended and the large bowel is irregularly ballooned at various parts. The ballooned areas occurred at the 4th inch from the anus, at the 8th inch, between the 9th and the 11th inches, between the 12th and the 14th inches, from the 16th to the 18th inches, and from the 18th inch up to the tip of the cæcum. The whole colon is 24" long from the tip of the cæcum to the neck of the bladder. The width of the cæcum at its widest part is  $1\frac{1}{2}$ ". The width of the colon at the dilated area opposite the 14th inch is one inch. In this case the ballooning is uniform, not unilateral. Fig. 3 C, shows this colon. The *stomach* is greatly dilated and but slightly congested. The first inch of the *duodenum* is much congested: below this, for a distance of one inch, the walls are excessively thin, almost transparent. The rest of the duodenum is but slightly congested and ecchymotic, its walls are very thin. The mucous membrane of the *small intestine* shows patchy ecchymoses here and there throughout the extent of the bowel, but they are not so marked as in previous cases; its walls are excessively thin. There is no marked colitis present. The lymphoid nodules at an area of mid-colon about 4" long are very prominent.

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# THE PATHOGENESIS OF DEFICIENCY DISEASE.

## No. VIII.—THE GENERAL EFFECTS OF DEFICIENT DIETARIES ON MONKEYS.

BY

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IN another place I have recorded the effects of certain deficient and ill-balanced dietaries on the gastro-intestinal tract of monkeys<sup>(1)</sup>. It is the purpose of the present paper to summarize their general effects, reserving for a future report an account of the histo-pathological changes in the internal organs.

The dietaries employed were the following :—

A. A control diet consisting of wheaten bread, plantains, milk, fresh onions, and ground-nuts. Nine monkeys were fed throughout the course of the experiment on this dietary.

B. An exclusive diet of autoclaved rice : that is to say, one deficient in suitable protein, in fat, in accessory food factors of all three classes and excessively rich in starch. Ten monkeys were fed to the point of death on this diet<sup>(1)</sup>.

C. A diet of autoclaved rice and butter : that is to say, one deficient in suitable protein, in accessory food factors of the 'B' and 'C' classes and excessively rich in starch as well as in fat. Four monkeys were fed to the point of death on this diet<sup>(1)</sup>.

D. A diet of autoclaved food (rice, wheaten bread, milk, and ground-nuts) to which a small ration of fresh onion was added : that is to say, a diet deficient in accessory food factors of the 'A' and 'B' classes. Six monkeys are included in this category.

E. A diet of autoclaved food (rice, wheaten bread, milk, and ground-nuts) to which fresh onion and fresh butter were added : that

is to say, a diet deficient only in accessory food factors of the 'B' class and excessively rich in fats. Five monkeys are included in this category.

Attention is directed to the composition of these dietaries. Their balance, or lack of balance, with respect to proteins, carbohydrates, and fats requires consideration as much as does their deficiency in vitamins. We shall see from the results of the experiments that they emphasize the importance of studying the 'dietetic history' in every case of gastrointestinal disorder or of vague ill-health in the human subject. And in so doing it would seem to be essential not to dissociate any existing deficiency of vitamins from other obvious defects, such as an insufficient supply of proteins or an excess of starch or of fats. For, it is to be remembered that deficiency of vitamins of the 'B' class often runs parallel with the excessive richness of starch in a diet, especially so when the source of the starch is polished rice, white flour, white bread, sago, tapioca and many proprietary foods. A dietary excessively rich in starch thus often connotes a deficiency of 'B-vitamine.' Now we shall see from the findings presently to be recorded what the effects of deficiency of this vitamine are in monkeys, and from these we are justified in assuming in similar circumstances like results in man. In man, however, it is not with complete vitaminic deficiency that we have most often to deal in practice, but with incomplete vitaminic deficiency. For the former causes rapid dissolution and death, the latter protracted dissolution and disease. It will be noted, moreover, that 'neuritic' lesions are by no means the most important of the effects of deficiency of 'B-vitamine.' Unfortunately, the adjective 'anti-neuritic' has been attached to accessory food factors of the 'B' class, so that a deficiency of this factor suggests the onset of 'polyneuritis' or of 'beri-beri' as its sole results. These, however, are but the grosser and later manifestations of such deficiency. The changes in the nervous system to which want of this vitamine gives rise are not its most important effects with respect to time of onset, to frequency of occurrence, and to need for recognition. It is true that lack of 'B-vitamine,' associated as it usually is with an insufficient supply of protein and an excess of starch, may ultimately lead to degenerative changes in nerve cells, but of greater practical importance is it to recognize that this combination of defects in human dietaries may be expected to lead more early and more often to degenerative changes in, and to depression and failure of function of, those cells upon which the processes of digestion and assimilation are dependent.



It is possible that the depression of function so induced may be accountable for a number of the commoner gastro-intestinal disorders, to which no bacterial or parasitic label has so far been attached. However this may be, the present records are offered as a contribution to their study. It will surprise those who investigate the 'dietetic history' of cases of chronic gastro-intestinal disorder to find how many persons habitually subsist on 'bread and butter' and 'milk puddings.' Such a dietary is poor in 'B-vitamine' and in protein, as well as excessively rich in starch and fats. It is my own experience in India that the 'dietetic history' of European sufferers from chronic colitis, and of those suffering from chronic gastro-intestinal disorders, commonly reveals the fact that their food does not contain the requisite proteins or the due proportion of starch, fats, salts, water and vitamins necessary for the maintenance of health. This one cannot 'digest' vegetables, fruit or meat or 'never touches them in India,' that one can 'carry on only on farinaceous food.' So it is that the form of diet which they commonly adopt is that often most calculated to promote the very disorder from which they seek relief. As we shall see from a perusal of these records, the cardinal effects of deficient and ill-balanced dietaries in monkeys are gastro-intestinal disorder, dilatation of the stomach, gastritis, and colitis. They are likely to be the same in man. It did not surprise me, therefore, to find that a patient, who consulted me recently, and who for ten years has subsisted mainly on milk puddings, had a dilated stomach, air-locks in the small bowel which caused her great discomfort, delay in the passage of the intestinal contents, colitis, a tender caecal region, and an inefficient pancreas with glycosuria. The results of these experiments have helped me to visualize the changes which are likely to be occurring in the gastro-intestinal tract in such a chronic invalid. I desire, therefore, at the very outset of this report to emphasize the importance in practice of a study of the dietetic history in such cases believing, as I now do, that bacterial agencies are often but weeds which flourish in soils made ready for them by dietetic-defects, and believing also that in the fuller comprehension of the science of dietetics we shall understand more perfectly the beginnings of disease and more fully its therapy.

#### 1. DETAILS OF THE EXPERIMENTS.

1. The rice employed in these experiments was Bellary milled rice. It was picked by hand to remove all grains having portions of adherent pericarp. It was then soaked in several changes of cold water for



48 hours, with the object of extracting a proportion of water-soluble accessory food factors. Finally, it was drained and autoclaved at a temperature of  $130^{\circ}\text{C}.$  for *one-hour-and-a-half*. It has been found that prolonged autoclaving at a temperature of  $130^{\circ}\text{C}.$  is necessary in order to destroy effectually all accessory food factors present in milled rice. The autoclaving of rice in bulk at a temperature of  $130^{\circ}\text{C}.$  for three-quarters of an hour does not appear to be sufficient to destroy all the 'B-vitamine' which it may contain. Pigeons fed on rice so autoclaved survive longer than do those fed on rice autoclaved at the same temperature for double the length of time. (Rice in bulk forms in the autoclave a glutinous mass which is of low permeability. No doubt, if it were spread out in thin layers in the autoclave, a temperature of  $130^{\circ}\text{C}.$  for three-quarters of an hour would be sufficient. Such a procedure would, however, require very large autoclaves.)

2. The autoclaved food employed in the experiments consisted of bread made from wheaten flour, milk, ground-nuts, and Bellary rice. These articles were subjected in the autoclave to a temperature of  $130^{\circ}\text{C}.$  for *one-and-a-half hours*—a treatment which, it may be presumed, completely destroyed their 'vitaminic' ingredients. Fresh butter and fresh onions, when added to food so autoclaved, provided accessory food factors of the 'A' and 'C' classes and other substances of nutritive value. The added butter, however, rendered the dietary, for wild monkeys, disproportionately rich in fats—a circumstance which was not without influence on the animals so fed.

3. The scale of rations provided for the monkeys in the five categories was as follows:—

- (a) (Controls) bread, 120 grams; milk, 120 c.c.; ground-nuts, 10 grams; fresh onion, 5 grams; fresh butter, 2 grams; plantains, 2; water.
- (b) Autoclaved rice, 120 grams; water.
- (c) Autoclaved rice, 120 grams; fresh butter, 15 grams; water.
- (d) Autoclaved bread, 60 grams; autoclaved rice, 60 grams; autoclaved milk, 120 c.c.; autoclaved ground-nuts, 10 grams; fresh onion, 5 grams; water.
- (e) Autoclaved bread, 60 grams; autoclaved rice, 60 grams; autoclaved milk, 120 c.c.; autoclaved ground-nuts, 10 grams; fresh onion, 5 grams; fresh butter, 15 grams; water.

The ration supplied to the animals in categories (b), (c), (d), and (e) was the maximum amount a healthy monkey would eat. It was found

that a monkey in vigorous health could not consume more than 120 grams of autoclaved rice daily. This amount was therefore fixed as the ration for animals in categories (b) and (c). When their appetites began to fail they consumed only a portion of it.

4. The monkeys (*macacus sinicus*) were captured in the jungles of Madras. The experiments were commenced within a fortnight of their capture. Those which were fed exclusively on autoclaved rice were for the most part adolescent animals. Those fed on autoclaved rice and butter were adults, as were the controls and also those fed on autoclaved food, butter, and onion. Of those fed on autoclaved food and onion three were adults while three were young, two of the latter still being with their mothers, although well beyond the stage of suckling. In both instances the mother and her young were confined in the same cage and received the same food (Nos. 21 and 22, 25 and 26).

5. The dimensions of the cages used were 24"×15"×14". Very little movement was, therefore, possible. Minute attention was paid to the general hygienic conditions under which the animals lived. When necessary the animal room was heated to an appropriate temperature by night, and the animals were protected from cold and draughts by thick curtains drawn at night around the shelves on which their cages were placed. These precautions were necessary since the monkeys, whose natural habitat was the warm moist climate of the East Coast of India, were extremely susceptible to cold at an altitude of 6,000 ft. above sea-level. The precautions were successful in preventing the death of any animals from causes attributable to climatic conditions. Two only were excluded for causes other than those directly attributable to the deficient dietaries. In one case an animal died on the seventh day of feeding on autoclaved rice; the cause of death was peritonitis. It is possible that it was injured during capture. A second animal amongst the controls was found at autopsy to have cystic disease of the kidneys and extensive consolidation of the lungs, these organs weighing 15 grams per kilo of original body-weight as compared with 6 grams in healthy animals. It was notable, however, that this monkey even after 118 days' confinement, under experimental conditions, showed no evidence of colitis or gastro-intestinal disorder. Neither of these animals is shown in Table I.

6. Three cases (Monkeys Nos. 9, 25, and 26) included in the autoclaved food and onion category, require special mention. Monkey No. 9

was fed for 28 days exclusively on autoclaved rice. Towards the end of this period it suffered from loss of appetite, vomiting, and diarrhœa. It exhibited also marked 'weakness' of the right leg. It was decided to attempt to prolong its life: its dietary was consequently enriched with wheaten flour bread. This it seized upon greedily, refusing to eat the autoclaved rice. Its health improved markedly, it looked much better and the diarrhœa ceased. At the end of one week during which it had received wheaten flour bread, it was placed on dietary (*d*), *i.e.*, on autoclaved wheaten bread, rice, milk, and ground-nuts, with a small ration of fresh onion. It survived this dietary for a further period of 52 days when it died. The right leg became more obviously affected as the experiment progressed so that the animal kept it constantly flexed and refrained from placing it on the floor of its cage. Towards the end of the experiment it became excessively anæmic, losing its appetite a week to ten days before death, and finally died of asthenia. Marked degeneration of peripheral nerve fibres was found on microscopical examination. Monkeys Nos. 25 and 26, mother and young, were fed for 16 days exclusively on autoclaved rice, and subsequently to the end of the experiment on autoclaved food and onion.

7. Monkeys Nos. 28, 31, 32, 33, and 34 were fed for the first ten days on autoclaved rice and butter, and subsequently on autoclaved food with butter and onion.

## II. DURATION OF THE EXPERIMENTS.

In the following table the duration of the experiments, the dietary employed in each case, the sex of the animals, and their initial and final body-weights are shown:—

TABLE I.

No. of monkeys.	Sex.	Original body-weight in grams.	Final body-weight in grams.	Percentage gain or loss in weight.	Days under experiment.	Dietary employed.
Controls	13 Female	2300	2300	± 0	33	Fresh bread, milk, onion, plantains, ground-nuts, water.
	15 "	2680	3050	+13	92	
	16 "	2740	2300	-17	106	
	17 Male	1780	1900	+ 6	106	
	18 "	2320	2170	- 7	104	
	19 Female	2420	2200	-10	104	
	20 Male	2470	2420	- 2	101	
	21 "	2170	2120	- 3	101	

TABLE I.—(Contd.)

No. of monkeys.	Sex.	Original body-weight in grams.	Final body-weight in grams.	Percentage gain or loss in weight.	Days under experiment.	Dietary employed.
Experimental animals	1 Male	2000	1500	-25	19	Autoclaved rice; water.
	2 Female	1350	950	-30	26	
	3 Male	1470	1040	-30	22	
	4 Female	1570	1170	-26	22	
	5 Male	1900	1400	-27	24	
	6 "	1380	980	-29	19	
	7 Female	1930	1450	-25	26	
	8 "	1650	1320	-20	26	
	11 Male	1900	1470	-23	22	Autoclaved rice and fresh butter; water.
	12 "	1780	1420	-21	28	
	27 Male	1810	1250	-31	15	
	29 Female	2130	1720	-20	13	
	30 "	2520	1850	-27	14	Autoclaved * food and fresh onion; water.
	36 Male	2410	1800	-26	18	
	9 Male	2090	1320	-37	82	
	21 "	1280	900	-30	67	
	22 Female	1850	1200	-36	67	Autoclaved * food, fresh butter and onion; water.
	25 "	1190	850	-29	62	
	26 "	2760	1820	-35	51	
	35 Male	1320	1050	-21	98	
	28 Male	2720	1700	-38	100	Autoclaved * food, fresh butter and onion; water.
	31 "	2250	1410	-38	71	
	32 Female	2400	1950	-19	51	
	33 Male	2030	1590	-22	43	
	34 Female	2710	1900	-30	80	

\* See paragraphs 6 and 7 above.

It is seen from this table, (a) that monkeys fed exclusively on autoclaved rice died, or reached the point of death, in periods ranging from 19 to 28 days, or in an average period of 23.4 days; (b) that monkeys fed on autoclaved rice plus butter died, or reached the point of death, in periods ranging from 13 to 18 days, or in an average period of 15 days; (c) that monkeys (Nos. 21, 22, 25) fed on autoclaved food plus a small ration of fresh onion died, or reached the point of death, in periods ranging from 67 to 98 days, or in an average period of 77.3 days; (d) that monkeys fed on autoclaved food plus fresh butter and onion died, or reached the point of death, in periods ranging from 43 to 100 days, or in an average period of 65 days; (e) that control monkeys fed on a well-balanced dietary suffered, as a rule, no ill effects other than those contingent on confinement in small cages. It appears, therefore, (1) that monkeys (*macacus sinicus*) cannot sustain life for much longer than 30 days on an exclusive diet of rice which has been

autoclaved at a temperature of 130°C. for 1½ hours; (2) that the addition of fresh butter to the dietary of rice so autoclaved hastens the death of the animals; (3) that deficiency of protein and excess of starch play an important part in hastening the death of the animals; and (4) that life cannot be sustained in monkeys, of the species employed, for much longer than 100 days in the almost complete absence of accessory food factors of the 'B' class, other essential requisites of an adequate dietary having been provided.

Now to my mind the last fact has a great significance: it suggests that it is not so much with complete lack of 'B-vitamine' that we, as physicians, have to deal in practice, as with its subminimal supply. Complete deprivation of this accessory food factor means comparatively rapid death; subminimal supply means subminimal function on the part of all organs and tissues and of the gastro-intestinal tract in particular. It requires a modicum of this substance to sustain life at all and such a subnormal existence constitutes in itself a state of disease. Complete vitaminic deprivation is a comparatively sudden dissolution, incomplete vitaminic starvation a slow and protracted dissolution. Indeed experimental research provides abundant proof of this conception, for a rice imperfectly polished or imperfectly autoclaved, and containing traces only of this vitamine, will sustain life for longer periods than a rice devoid of vitamine, but in the end the inevitable dissolution is the same.

### III. LOSS OF BODY-WEIGHT.

As the animals were exceedingly wild and difficult to handle they were weighed only at the beginning and at the end of the experiments. The average loss of weight in each category is shown in the following table:—

TABLE II.

Category and number of animals in each.	Average weight before experiment, in grams.	Average weight after experiment, in grams.	Average loss of weight per kilo. of original body-weight, in grams.	Average days under experiment.	Average daily loss of weight, in grams, per kilo. of original body-weight.
(a) Controls, 8 ..	2360	2307	22	93·5	0·23
(b) Autoclaved rice, 10 ..	1693	1270	249	23·4	10·6
(c) Autoclaved rice and butter, 4 ..	2217	1655	253	15	16·8
(d) Autoclaved food and onion, 6 ..	1748	1190	320	71·1	4·5
(e) Autoclaved food, butter and onion, 5	2422	1710	294	65	4·5

From this table it is seen that the controls maintained a fairly constant level of weight throughout an average period of 93·5 days; individuals, however, showed varying moderate losses or gains in weight (Table I), the most notable being No. 16 which lost considerably. The relatively slight average loss of weight in the controls as a whole is attributable to the fact that sudden confinement of wild animals cannot fail to influence them unfavourably. The comparatively high level of health maintained by the controls is sufficient evidence of the appropriateness of their food-supply. In no case did a control animal exhibit symptoms of gastro-intestinal disorder apart from a tendency to constipation. During the earlier part of the experiment they received a too generous supply of ground-nuts with the result that some of them developed jaundice. This symptom disappeared in the course of a few days on discontinuing the ground-nuts, limiting the dietary to milk and plantains, and adding to the milk a pinch of magnesium sulphate.

It will be noted that in all four categories of deficiently fed animals the loss of weight ranged between 25 and 32 per cent, the most rapid loss of weight occurring in animals fed on autoclaved rice and butter. The table emphasizes the harmful influence exercised by the addition of the butter to a dietary excessively rich in starch and deficient in proteins and accessory food factors. It emphasizes also the important part played by an adequate supply of suitable protein in prolonging the life of the animals.

#### IV. CLINICAL OBSERVATIONS.

Monkeys in all four categories presented in general the same clinical features: progressive anæmia, asthenia, and gastro-intestinal disturbances. These, indeed, are to be regarded as the cardinal symptoms of the morbid state induced by the deficient dietaries. Some animals exhibited other clinical features which will be referred to under their appropriate heading. The essential difference in the four categories was the greater or lesser degree of rapidity with which these symptoms manifested themselves, and the greater or lesser delay in the inevitable issue—death.

The animals were unfortunately too wild to handle, so that it was found impracticable to make extended observations with regard to respiration and body-temperature. Such as were made towards the end of the experiment indicated that the temperature was usually subnormal and the pulse weaker and slower than in health.

*Anæmia* made its appearance rapidly in animals fed on autoclaved rice, more slowly in those fed on autoclaved food. Judged by the





PLATE XLII.



Fig. 1.—Monkey No. 26, fed for 16 days on autoclaved rice and subsequently on autoclaved food (bread, milk, ground-nuts and rice) with fresh onion. Photograph taken on the 50th day of experiment. Note wrist drop. Femoral nerves showed moderate degree of degeneration.

intense pallor of the face, the degree of anæmia in animals fed on autoclaved food must have been pronounced. I was unable to undertake blood examinations.

*Asthenia* was gradual in its onset in monkeys fed on autoclaved food, rapid in its onset in those fed on autoclaved rice. Towards the end of the experiment, the degree of asthenia amongst animals in all categories was profound. *Weakness of the limbs* was, as a rule, well marked towards the end of the experiment in all animals. I was, however, unable to satisfy myself, by clinical methods of study, that this weakness was due to 'neuritis.' Examination of the peripheral nerves after treatment by Marchi's method revealed, however, a high proportion of degenerative change in animals fed on autoclaved food. No doubt, the weakness of the limbs was due in some part to these lesions (page 335). Monkeys exhibiting leg symptoms were observed to limp, one leg being kept flexed and held away from the ground, giving the impression of pain in the limb. It was noted also in more than one instance that the right leg would be so held on one day, the left on another. Towards the end of the experiment, when they were made to move about the floor of the laboratory, it was often observed that one limb tended to give way under the monkey's weight. This symptom applied to both upper and lower limbs, so that animals so affected often toppled over on their sides and regained the erect position laboriously; such was the case in the animal shown in Fig. 1. Sometimes the lower limbs assumed a spread-eagle appearance and could only be drawn under the body with difficulty. As a rule, animals fed on autoclaved rice or on autoclaved rice with butter died before any noteworthy clinical symptoms other than intense weakness were observable in their limbs. The proportion of these cases exhibiting degenerative changes in the peripheral nerves was found to be small (page 335).

An appearance suggestive of *wrist-drop* (Fig. 1) was sometimes noted in animals fed on autoclaved food. The hands of healthy monkeys are often held in the dependent position shown in the figure, but while in health this position appeared to be one of choice, in the diseased animals it was often one of necessity. An animal, seated as in the figure, would be observed to interest itself in the capture of body-fleas, all movements to this end being carried out with the wrist in the 'wrist-drop' position. In this pursuit it seemed to be unaware that the tips of the first finger and thumb were not in apposition, and to be greatly surprised when the object of its search was not found between them.

Two monkeys, fed on autoclaved food, devoted a great deal of their time, before they became too weak to do so, to the capture of flies which they ate greedily. One animal, especially skilful in this pursuit, survived for as long as 100 days on the autoclaved food-butter-onion dietary. No doubt they were aware of some want in the food and employed this device to remedy it.

*Headache* appeared to be a fairly constant symptom, if one was justified in assuming its presence by the attitudes taken up by the animals: they were often observed to support the head in the hand as though in pain. Those fed on autoclaved food almost always presented towards the close of the experiment a dazed expression, suggestive of wonder or surprise at the feeble state in which they found themselves.

*Gangrene of the tail* was met with in a number of cases.

*Diminished sensibility* was undoubtedly present in the majority of animals in all four categories towards the end of the experiment. It was often noted as regards the tail, that the animals seemed to be quite unaware of the fact that one had placed one's foot upon it. I have often tapped the tail sharply with a ruler from the tip to the root without the animal appearing to be aware of my action.

While then I am unable to say on clinical grounds that any of these animals suffered from 'polyneuritis,' it was obvious that, in the autoclaved food class, the majority of them, during the later days of the experiments, exhibited a pronounced depression of functional capacity of the nervous system; this conclusion was confirmed by subsequent examination of the peripheral nerves.

*Gastro-intestinal disorders.*— These have been dealt with at length in another place in so far as they were observed to affect monkeys fed on (a) autoclaved rice, and (b) on autoclaved rice and butter<sup>(1)</sup>. To this account the reader is referred. In character these disorders were the same in all four categories although differing in incidence or degree. It will suffice, therefore, to summarize the clinical observations under the heading in so far as they refer to the remaining two categories, i.e., (a) those fed on autoclaved food and onion, and (b) those fed on autoclaved food with butter and onion.

*Loss of appetite* made its appearance much later in the two classes of animals fed on autoclaved food than in those of the two classes fed on autoclaved rice. Indeed, the appetite remained, as a rule, fairly good in the former up to within seven to ten days of the fatal issue.

*Vomiting* was observed in only one animal fed on autoclaved food and onion. In this case marked congestion of the stomach was found at autopsy, the mucous membrane of the pyloric half of the organ being especially congested, and ecchymotic.

*Diarrhœa*.—This symptom was present in four out of six monkeys fed on autoclaved food and onion. Amœbæ, but without ingested red blood corpuscles, were present in two cases.

*Dysentery*.—This symptom was present in one animal fed on autoclaved food and onion, and in four fed on autoclaved food with butter and onion. Amœbæ, having ingested red blood corpuscles, were present in two cases.

Of eleven animals, therefore, the basis of whose dietary was autoclaved food, nine had either diarrhœa or dysentery. In eight of these colitis was a prominent feature at autopsy. It occasionally happened that in a given animal diarrhœa was present at some time during the earlier days of the experiment and dysentery during the later days. Thus in monkey No. 21, fed throughout the course of the experiment on autoclaved rice plus onion, diarrhœa, which was blood-stained but without amœbæ, made its appearance on the twenty-second day and persisted for several days. The symptom then cleared up only to reappear as a grave dysentery 39 days later. The dysentery persisted until the animal was killed on the 67th day of the experiment. No pathogenic amœbæ were present.

*Dropsy*.—No clinical evidence of dropsy was met with in any of the four categories.

*Disorders of the skin*.—Disordered function of the skin was evidenced chiefly by the changes which occur in the hair. It became coarse and staring (Fig. 1) and showed a marked tendency to fall out. The skin was often rough and scaly and in some cases a scaly eczematous condition was present. It seemed also that the skin was less sensitive than usual. A bleeding eczematous condition of the parts surrounding the anus was observed in two monkeys. The coarse staring coats of guinea-pigs, fed on a scorbutic dietary, and the ease with which the hair falls out will be familiar to all who have used guinea-pigs for 'scorbutic' experimental work. It will be remembered too that acniform eruptions of the skin and seborrhœic eczema are sometimes amongst the most distressing of the disabilities from which the subjects of chronic colitis suffer.

#### V. MORBID ANATOMICAL OBSERVATIONS.

The procedure adopted in the case of pigeons, described in detail in a previous communication<sup>(2)</sup>, was followed also in monkeys. They were, as a rule, autopsied immediately after death, and the heart's blood

examined by aerobic methods of culture. All organs were weighed with the least possible delay after their removal from the body.

A. *Hæmic infections*, as demonstrable by aerobic methods of culture, were present as follows:—

Control animals (8) .. .. .	Nil.
Autoclaved rice (10) .. .. .	4
Autoclaved rice and butter (4) .. .. .	1
Autoclaved food and butter (6) .. .. .	1
Autoclaved food, butter, and onion (5) .. .. .	Nil.

The organisms were mainly of two kinds, as determined by microscopical examination: small cocci and coliform bacilli; no attempt, however, was made to differentiate them.

These findings would appear to indicate that autoclaved food with onion, or with butter and onion, is less favourable to the occurrence of hæmic infections than is a dietary composed mainly of carbohydrates and deficient in vitamins.

B. *Weights of organs*.—The weights of the organs, in grams per kilo of original body-weight\*, in controls and in animals of all four categories, are shown in the accompanying table:—

TABLE III.

Organs.	Controls. 8	Autoclaved rice. 10	Autoclaved rice and butter. 4	Autoclaved food and onion. 6	Autoclaved food, butter, and onion. 5
Adrenals ..	0.190	0.352	0.293	0.258	0.266
Adrenalin ..	0.000301	0.000393	0.000264	0.000224	0.000224
Thyroid ..	0.083	0.081	0.073	0.069	0.070
Pituitary ..	0.014	0.015	0.015	0.016	0.0118
Testicles ..	0.257	0.216	0.218	0.208	0.254
Thymus ..	0.54	Traces only	Traces only	Traces only	Traces only
Sub-maxillary glands ..	1.01	0.80	0.70	0.82	0.78
Pancreas ..	1.36	1.14	0.95	1.22	1.07
Spleen ..	1.5	1.3	1.1	1.2	0.9
Liver ..	29.8	26.4	27.8	22.2	25.3
Heart ..	4.47	3.53	3.44	3.03	3.8
Kidneys ..	5.03	5.16	5.3	4.6	4.8
Brain ..	28.8	41.5	32.4	37.0	29.8
Lungs ..	6.03	5.5	4.9	5.1	5.2

*Note*.—The adrenalin-content of the suprarenals of monkeys fed on autoclaved rice was 0.000393 gm. in non-infected animals, 0.000264 gm. in infected animals.

\* Original body-weight was taken as the basis for these calculations since it appears to afford a fairer criterion of the degree of departure from health. When calculated against the final body-weight of the animals the results are, of course, much more marked.—R. McC.

From a study of this table and the figures (4, 5, 6, and 7) which illustrate it, it will be seen that the organs group themselves into three classes :—

A. Those which increase in weight in all four categories :

The Adrenals.

„ Brain.

B. Those which decrease in weight in all four categories :

The Thymus.

„ Heart.

„ Sub-maxillary Glands.

„ Pancreas.

„ Spleen.

„ Liver.

„ Lungs.

„ Thyroid.

„ Testicles.

C. Those which show an increase in weight in some categories and a decrease in others :

The Pituitary.

„ Kidneys.

The similarity of these changes in weight of the organs of monkeys to those previously reported in pigeons is very striking<sup>(2,3)</sup>. In kind they are the same, although in degree they may differ. There is the same enlargement of the adrenals and pituitary and the same atrophy of the thymus, the pancreas, the heart, and the thyroids. The atrophy of the testicles and spleen is much more intense in birds, while the increase in weight of the brain observed in monkeys whose dietary was excessively rich in butter was observed only in pigeons which received butter in addition to autoclaved rice<sup>(3)</sup>. The lungs alone presented any material difference in the two species : in pigeons fed on autoclaved rice and butter, an increase in the weight of the lungs was noted<sup>(3)</sup> ; whereas in monkeys in all four categories, these organs showed a marked reduction in weight.

Now although neither the increase in weight of an organ on the one hand, nor its decrease in weight on the other, can *per se* be regarded as conclusive evidence of increased or of diminished functional capacity,



nevertheless notable alterations in weight, when taken in conjunction with histological changes, are important indications of disordered functional capacity.

The extent to which the various organs were affected by the dietetic deficiencies may now be considered.

C. *The salivary glands.*—These organs, as represented by the sub-maxillary glands, undergo some degree of atrophy in consequence of all the deficient dietaries employed (Fig. 5). The atrophy was most marked in animals fed on autoclaved rice and butter, that is to say, in animals whose dietary was excessively rich in starch and fat and deficient in protein and accessory food factors of the 'B' and 'C' classes. It is notable that in each category the salivary glands may be more affected in one animal than in another. It seems probable that impairment of function of these organs may be regarded as one result of dietetic deficiency, and that the process of digestion is thus impeded at its very onset.

D. *The stomach.*—Dilatation of the stomach is a very common consequence of food deficiency (Figs. 2, 3). It was invariably present in animals the starchy components of whose food were in great excess over other essential constituents. It was less marked in animals fed on autoclaved food (*vide* Table IV). The mucous membrane of the stomach was frequently congested and ecchymotic especially in the region of the pylorus. Shallow ulcers in this locality were occasionally encountered (Table IV). Deficiency of accessory food factors alone, other components of the food being supplied in adequate quantity, is capable of giving rise to these congestive changes in the stomach although less frequently than in the presence of an excess of starch in the dietary. It will be noted from a study of Table IV that in the former circumstances gastric catarrh was present in five out of eleven cases, in the latter in ten out of fourteen cases. A notable finding in the case of monkey No. 32, fed on autoclaved food with butter and onion, was a small area of recently developed carcinoma of the pylorus visible only on microscopical examination of sections (page 342.)

E. *The duodenum.*—Pronounced congestion of the duodenum was a frequent result of the imperfect dietaries employed in these experiments. The congestion was often so marked as to extend to the serous coat of the bowel (Table IV). Such duodenal catarrh has been found in animals whose dietary was deficient only in accessory food factors as well as in those in which the carbohydrate element of the food was excessive;





PLATE XLIII.

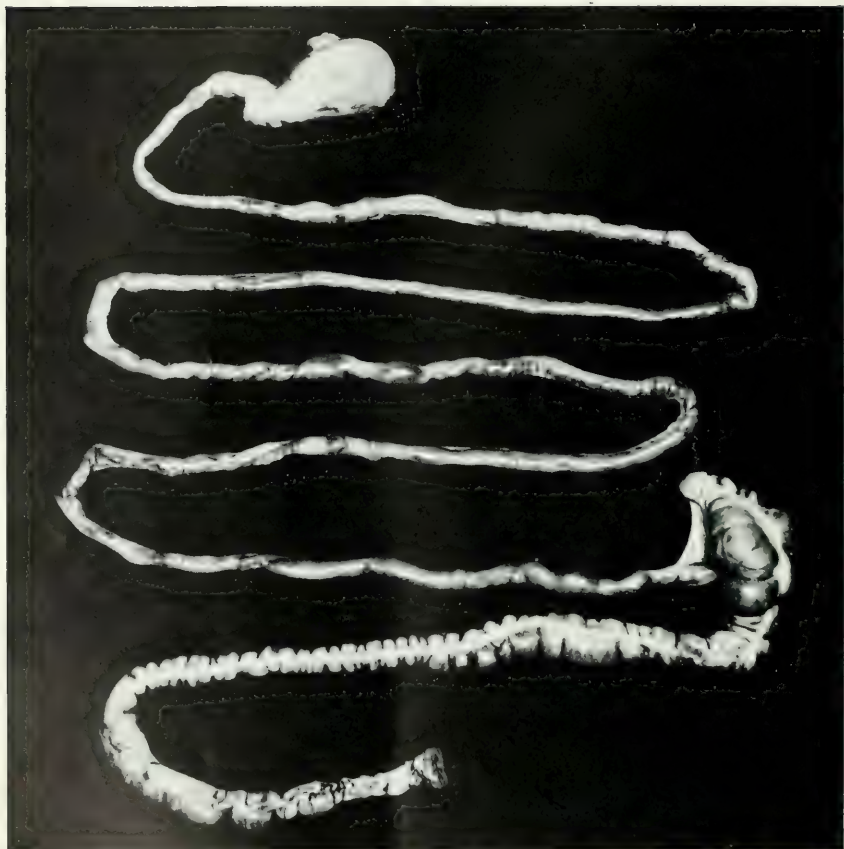


Fig. 2.—Photograph of gastro-intestinal tract of control monkey No. 18. Killed on 104th day of the experiment 15 hours after last meal. Stomach and mid-colon empty, cecum loaded, lower bowel contains small amount of fecal matter. Bowel evacuated before death. Same scale as Fig. 3, with which compare.

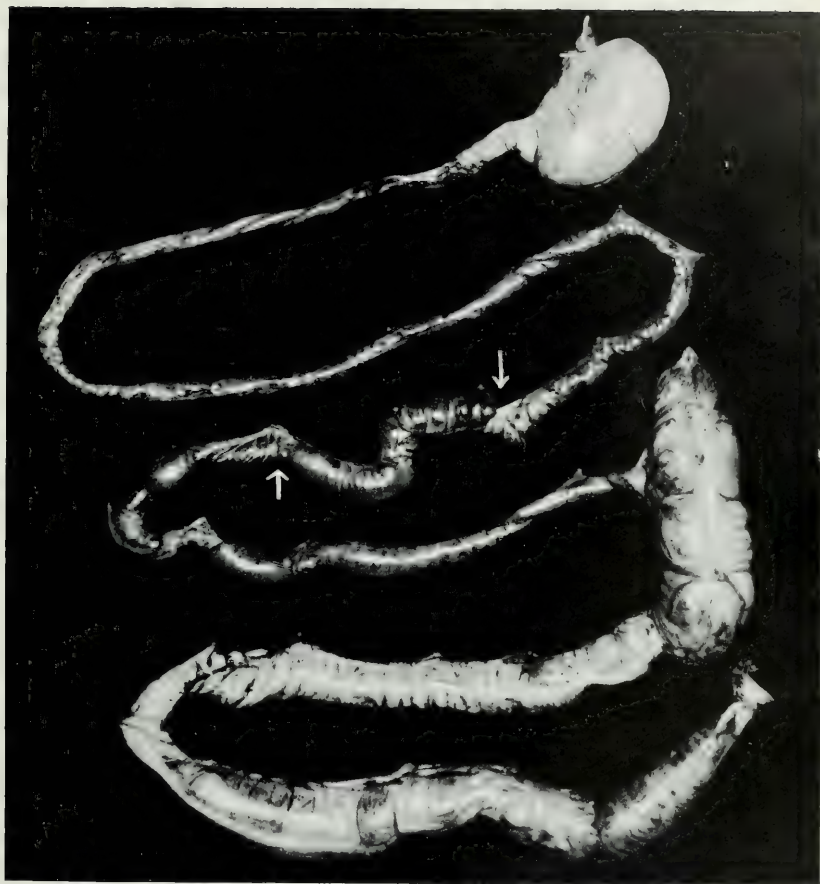


Fig. 3.—Photograph of gastro-intestinal tract of monkey No. 22, fed exclusively for a period of, 67 days on autoclaved food and onion. The animal (an adult female) died on the same day as her young which was similarly fed. Note dilatation of empty stomach, also of cecum and large bowel. Large ileal intussusception is indicated by arrows. Note inert appearance of greatly dilated colon. Intense colitis was present throughout the whole length of the viscus. The sub-serous ecchymosis especially at lower part of the colon can just be made out in the photograph. Compare with Fig. 2.



its incidence in the latter was much higher than in the former (Table IV), being least marked in animals whose dietary was deficient only in the 'B-vitamine.' Ulcer of the duodenum has not been met with in monkeys although it has been observed in guinea-pigs fed on a scorbutic dietary<sup>(4)</sup>. Thinning of the muscular coats of the duodenum and necrotic changes in its mucous membrane were also frequent accompaniments of dietetic deficiencies, but, as a rule, only when there was great excess of starch in the dietary.

F. *The small intestine.*—There is a remarkable tendency to intussusceptions in this organ which appears to be due in some measure to the dietetic defects. It is notable in this connexion that the increase in cases of intussusception amongst the ill-fed German population during the late war has been commented upon by German physicians<sup>(5)</sup>. The tendency to extensive intussusceptions was less marked in monkeys fed on autoclaved food (Fig. 3) than in those fed on autoclaved rice. Small invaginations were as frequently encountered in the latter, but they were, as a rule, obviously agonal in origin. Great distension of the small bowel leading to ballooning of certain areas and to 'air-locks' (the cause of much discomfort in certain gastro-intestinal disorders in human beings) is a common manifestation of dietetic deficiency. It was especially notable in animals whose food was excessively rich in starch and was, no doubt, the result of acid-fermentation in the small bowel. Enteritis was frequently present, sometimes involving the whole of the small bowel, sometimes limited areas only. The congestion of the bowel may even extend to the serous coat. It is notable that in animals fed on autoclaved food the small bowel was very little affected as regards congestive changes (Table IV), and that in general it suffered much less than in animals whose dietary was excessively rich in starch. In butter-fed animals whether receiving autoclaved rice or autoclaved food a peculiar yellow-white tinge was frequently imparted to the whole gastro-intestinal tract.

G. *The colon.*—Colitis is one of the most common and most constant results of dietetic deficiency. It was present in every case except one in three out of the four categories in monkeys. Its incidence, although high, was notably less in animals fed on autoclaved food, butter, and onion (Table IV). The colitis was frequently very extensive and small ulcers with adherent sloughs were often encountered in colons of animals fed on autoclaved food. In some cases it involved the whole colon from the cæcum downwards, the serous coat being often

ecchymotic. More commonly it was limited to the lower part of the great bowel, while occasionally a patchy colitis was present affecting limited areas, usually of the mid-colon. In one control animal (No. 16), a small area of the mid-colon was congested giving to the mucous membrane a rose-pink tinge, but without erosion of the mucous membrane or ecchymosis of the serous covering of the bowel. The animal was constipated and had suffered from jaundice. In no control animal was colitis present. The colitis was frequently accompanied with amœbic infection; the infection in some cases was due to *Entamœba histolytica*(<sup>1</sup>), in others to *Entamœba coli*. Ballooning of the cæum and colon with atrophy of the longitudinal muscular bands and loss of the normal puckerings of the great bowel were common appearances in all categories (Figs. 2 and 3). These appearances are suggestive of impairment of its neuro-muscular control. It may here be noted that a diffuse, apparently cancerous, infiltration of the submucous coats of the lower end of the great bowel was found in one monkey fed on autoclaved food, butter, and onion. The bowel wall was at this area much thickened and tough to the scissors. The mucous surface presented the appearances seen in granular ophthalmia. On histological examination occasional fine epithelial projections into the submucous coat were noted, as well as groups of epithelial cells scattered throughout a fibrous infiltration of the submucous coat.

H. *The pancreas.*—Varying degrees of atrophy of the pancreas were constant consequences of the deficient and ill-balanced dietaries (Fig. 5). In each of the four categories some animals were more affected in this regard than others. The atrophy was most marked when the diet was deficient in the 'B' and 'C' classes of vitamins and at the same time excessively rich in fats and in starch as well as poor in protein (Table III). It was notable also that the atrophy was very considerable in animals whose dietary, while excessively rich in fats, was deficient only in 'B-vitamin.' This effect of excess of fat in increasing the degree of atrophy of the pancreas has been recorded also in pigeons(<sup>3</sup>). It is difficult to resist the conclusion that an excessive fat intake, in the presence of vitaminic deficiency, is responsible for the more pronounced atrophic changes in the pancreas in these circumstances. Although anticipating a more complete account of the histo-pathology of the pancreas, to be published at a later date, it may here be said that the histological changes in this organ were as indefinite in monkeys as they were in pigeons(<sup>6</sup>). Apart from a diminution in size and shrunken appearance

of the alveolar cells, and their less constant exhibition of the characteristic darkly staining outer and lightly staining inner zone, I have been unable definitely to detect any gross histological change with this exception: the alveolar cells in the atrophic organs were not found to exhibit in the same degree that loading with granular contents which is characteristic of the cell before secretion, and indicative of healthy functional activity. I have examined serial sections of the pancreas from all 33 monkeys employed in these experiments, but pancreatitis was never encountered. Occasionally small hæmorrhages into the organ were present. To the naked eye the pancreas was usually pale in colour and of a consistency softer than in health. Although I cannot give a more definite account of the histological changes in the atrophic organ, the impression left by their study is that the function of the pancreas was in general considerably impaired.\* I have found no marked changes in the islets of Langerhans; they appeared to be increased in numbers and to form a greater proportion of the section in animals fed on autoclaved rice. In these the element of inanition may have been responsible for this appearance. It seems probable that these findings, *viz.*, (a) atrophy of the pancreas as estimated by weight, (b) its greater atrophy when the diet is deficient in 'B-vitamine' and protein and excessively rich in fats and starch, and (c) the histological appearances suggestive of diminished functional capacity, may have an important bearing on the genesis of diabetes in Bengalis. A dietary of autoclaved rice and butter, such as those monkeys received whose pancreas was the most atrophic, is similar in many respects to that commonly used by the diabetic Bengali. If to such a dietary an excessive sugar intake were added, its similarity would be the more marked. My findings with respect to the pancreas and the gastro-intestinal tract appear to be complementary to those of Colonel McCay and his colleagues whose work on the subject of diabetes was published in the April, 1919, number of this journal(7). Their conclusions as regards the gastro-intestinal changes, attributable to the highly carbonaceous dietary of the Bengali, find ample confirmation in the facts I have recorded. In my monkeys the urine was frequently aspirated from a full bladder at autopsy; its examination for sugar invariably yielded negative results. Blood sugar estimations were made in a number of animals fed on autoclaved food, butter, and onions, but in no case was hyper-glycæmia

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\* See account of histology of pancreas in polyneuritis avium, page 269—R. McC.



encountered.\* The changes induced in the adrenal glands by dietetic deficiency may not be without influence in regard to the genesis of diabetes.

I. *The liver.*—It will be noted from Table III that in all categories the weight of the liver per kilo of body-weight was considerably below that of health (Fig. 7). This was especially notable in animals fed on autoclaved food and onion. But it is difficult in animals of this category, as well as in animals fed exclusively on autoclaved rice, to form a just appreciation of the extent of this atrophy since in both of these categories the animals were, as judged by weight, considerably younger than the controls. One is not aware, therefore, of the normal weight of the liver per kilo in young animals. No such difficulty arises, however, in the case of monkeys fed on autoclaved rice and butter, nor in that of animals fed on autoclaved food, butter, and onion. Between these a just comparison is possible since the animals in these categories, as well as the control animals, were adults. It may be concluded then that lack of 'B-vitamine' alone can cause a notable degree of atrophy of the liver. It will be observed that in the two main categories—(a) those fed on autoclaved rice, and (b) those fed on autoclaved food—the atrophy was not so great when butter was added to the dietary. This result has previously been recorded for pigeons<sup>(3)</sup>.

Toxic absorption from the diseased gastro-intestinal tract will arise with the more ease in proportion as the liver is impaired in its function by the deficient dietary. The gall bladder was frequently observed to be distended in these animals.

For purposes of comparison I have prepared the following table showing the lesions of the digestive organs encountered at autopsy in monkeys fed on the various deficient dietaries :—

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\* My attention was unfortunately not directed to this aspect of the research until the majority of the monkeys had succumbed.—R. McC.

TABLE IV.

*Showing lesions of the digestive organs found in monkeys fed on the various deficient dietaries.*

Number of animals. Lesions.	Controls. 8	Auto- claved rice. 10	Auto- claved rice and butter. 4	Auto- claved food and onion. 6	Auto- claved food, onion, and butter. 5
Atrophy of sub-maxillary glands* ..	<i>Nil.</i>	6	4	5	2
Dilatation of stomach ..	<i>Nil.</i>	10	4	4	2
Gastric catarrh ..	1 (No. 16)	7	3	3	2
Ulcer of pylorus ..	<i>Nil.</i>	<i>Nil.</i>	1	2	<i>Nil.</i>
Carcinoma of pylorus ..	<i>Nil.</i>	<i>Nil.</i>	<i>Nil.</i>	<i>Nil.</i>	1
Duodenal catarrh ..	<i>Nil.</i>	7	3	2	1
Sub-serous ecchymosis of duodenum ..	<i>Nil.</i>	5	1	1	1
Duodenal ulcer ..	<i>Nil.</i>	<i>Nil.</i>	<i>Nil.</i>	<i>Nil.</i>	<i>Nil.</i>
Congestive changes in jejunum ..	<i>Nil.</i>	8	1	1	1
Congestive changes in ileum ..	<i>Nil.</i>	7	2	<i>Nil.</i>	<i>Nil.</i>
Sub-serous ecchymosis of jejunum ..	<i>Nil.</i>	5	<i>Nil.</i>	1	<i>Nil.</i>
Sub-serous ecchymosis of ileum ..	<i>Nil.</i>	3	1	<i>Nil.</i>	<i>Nil.</i>
Ballooning of small bowel ..	1 (No. 16)	6	4	2	2
Thinning of bowel walls ..	1 (No. 16)	10	4	2	4
Intussusception ..	<i>Nil.</i>	6	4	5	3
Colitis ..	<i>Nil.</i>	10	3	6	2
Sub-serous ecchymosis of whole colon ..	<i>Nil.</i>	2	<i>Nil.</i>	1	1
Sub-serous ecchymosis of lower colon only ..	<i>Nil.</i>	5	1	3	2
Ballooning of colon ..	1 (No. 16)	6	3	4	3
Atrophy of colon walls ..	<i>Nil.</i>	10	4	3	4
Enlargement of mesenteric glands ..	1 (No. 16)	10	4	6	4
Atrophy of omentum ..	<i>Nil.</i>	10	4	6	5
Atrophy of pancreas* ..	<i>Nil.</i>	6	2	4	2

\* Notes:—(1) Cases are noted as having atrophic sub-maxillary glands and pancreas when the weight of these organs was less than the lightest amongst controls.

(2) Intussusceptions are noted in every case in which they were present. The great majority of them were agonal in character. All were such in animals fed on autoclaved food, butter, and onion; all but one were such in animals fed on autoclaved food and onion (Fig. 3), while in other categories a considerable proportion as judged by their size and appearance had probably occurred some time prior to death.

The table brings into prominence the importance of excess of starch in accentuating the changes initiated by the dietetic deficiency. It would appear that it is to the excessive starchy component of the food that a considerable proportion of the disorder in the stomach and small bowel is attributable. It will be noted that while the stomach and the small intestine are adversely influenced by the excess of starch, the colon appears to suffer equally whether the deficient dietary is excessively

rich in starch or not. It is notable also with what constancy the omentum is devoid of fat in all categories. It seems to me not unlikely that the loss of the protective layer of omental fat is calculated to increase the susceptibility of the intestines to such influences as chill, enveloped as they are in health by the fat-laden omentum. Again the great constancy of enlargement, discolouration and infection of the mesenteric glands introduces the question of 'intestinal toxæmia.' These glands have yielded growths of coliform organisms and cocci by aerobic methods of culture. The absorption of poisonous products through the gastro-intestinal mucosa is greatly facilitated by an unhealthy state of its epithelium, and it is clear that deficiency of accessory food factors, especially when associated with deficiency of proteins and an excessive starch intake, leads to malnutrition, necrosis, and desquamation of the gastro-intestinal epithelium. The toxic products derived from the intestinal contents, and from the desquamated cells themselves, would, in these circumstances, find no difficulty in reaching the blood-stream in large amounts. Dietetic deficiency may then be regarded as a common cause of intestinal toxæmia, for such degrees of injury to the gastro-intestinal mucosa as may be necessary to admit of toxæmia from the bowels are readily produced by dietetic deficiencies. Then, too, the liver—that sentinel of the gastro-intestinal tract against toxæmia—suffers markedly in consequence of the deficient dietaries, and it seems probable that toxic products which in health are readily destroyed by it may in circumstances of dietetic deficiency be allowed to pass in considerable quantity.

1. *The adrenals.*—All classes of deficient dietary hitherto employed, whether in pigeons, guinea-pigs or monkeys, give rise to enlargement of the adrenal glands (Fig. 3). The adrenalin-content of the enlarged organs differs greatly with different classes of deficient and ill-balanced dietaries. It is dependent also to a very large extent on the presence or absence of hæmic infections: when hæmic infections were present the adrenalin-content *per gram of gland* was invariably low. I have previously reported (a) a low adrenalin-content in consequence of a scorbutic dietary in guinea-pigs, (b) a high adrenalin-content in consequence of an exclusive dietary of autoclaved rice in pigeons, (c) a low adrenalin-content in pigeons (with certain exceptions) fed on autoclaved rice plus butter. All these observations have reference only to animals in which no hæmic infection was demonstrated at autopsy by aerobic methods of culture. In the present experiments the adrenalin content was high in monkeys

fed exclusively on autoclaved rice whose blood was found to be sterile at the time of death, 0·000393 gram per kilo of original body-weight; low in monkeys fed on autoclaved rice and butter, 0·000224 gram per kilo of original body-weight (Table IV), thus confirming previous observations in pigeons. It was low also in monkeys fed on autoclaved food whether with or without butter. Although it is not possible at present to appreciate the true significance of these results, it is obvious that the nature of an animal's food has a profound influence on the adrenalin-content of the adrenal glands. When the pendulum of opinion as to the function or lack of function of the adrenal medulla has ceased to oscillate so vigorously, it will be found, I think, that the adrenal medulla is intimately concerned with healthy nutrition and with the normal metabolic processes of the body. Certain it is that a fruitful method of studying the function of this organ is to observe what happens to it in the varying phases of health, disease, imperfect food-supply and infection of the body. I have previously recorded the fact that in pigeons whose heart's blood yielded bacterial growths by aerobic methods of culture the adrenalin-content of the adrenal glands was invariably low<sup>(9)</sup>. The same result has been observed in monkeys (Table III).

In all animals in which these organs have been studied—pigeons\*, guinea-pigs, and monkeys—the left adrenal is larger than the right. In monkeys the right adrenal is per kilo of body-weight about one-seventh part lighter than the left. When, however, butter was added to a dietary either of autoclaved rice or of autoclaved food the difference in weight between the two adrenals was more marked. Then the right adrenal was from one-fourth to one-fifth part lighter than the left. Thus is provided another curious example of the influence of butter on the adrenal glands.

*Œdema.* In previous communications (<sup>2,3,9</sup>), I have drawn attention to the intimate association of adrenal hypertrophy with œdema. Unfortunately in the present experiments evidences of œdema were scanty:

These were as follows:—

- (a) Monkey No. 7 fed on autoclaved rice: 1 c.c. of fluid in the pericardium.

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\* In my first paper on the 'Pathogenesis of Deficiency Disease' published in the January, 1919, number of this Journal, I have stated, on page 304, with regard to the adrenals in pigeons that 'the right is commonly the larger of the two.' As the bird lies on the dissecting table the adrenal to the right of the observer is commonly the larger of the two, this is, of course, the left adrenal. I regret the error which I now correct.—R. McC.

- (b) Monkey No. 9 fed on autoclaved food with onion: 4 drops of fluid in the pericardium.
- (c) Monkey No. 33 fed on autoclaved food with onion and butter:  $\frac{1}{2}$  c.c. of fluid in the pericardium.
- (d) Monkey No. 34 fed on autoclaved food, butter, and onion: 5 c.c. of fluid in the abdominal cavity.

These findings are too scanty to admit of any conclusions being drawn with regard to them, especially so as three drops of fluid were present in the pericardial sac of one control animal.

K. *The pituitary.*—It will be observed that the pituitary of monkeys fed on autoclaved rice, on autoclaved rice and butter, and on autoclaved food increased in weight (Fig. 4). Thus is the finding previously reported in pigeons confirmed<sup>(2,3)</sup>. It is found also on analysing the figures further that the average weight of the pituitary per kilo of body-weight in healthy male monkeys of this species is 0.0133 gram, in healthy females 0.0152 gram. In health the organ is thus considerably heavier in females than in males, a finding which confirms previous observations in pigeons<sup>(3)</sup>. While, however, the pituitary enlarges in male monkeys, fed on autoclaved rice, to 0.015 gram per kilo of original body-weight, in females similarly fed it does not enlarge, being but 0.014 gram; a result which confirms that previously recorded for pigeons<sup>(3)</sup>. In these experiments a notable finding with regard to the pituitary was the marked drop in weight of this organ in monkeys fed on autoclaved rice with butter and onions. I have at present no explanation to offer of this observation.

L. *The thymus.*—In monkeys, as in pigeons<sup>(2,3)</sup>, the thymus atrophies sometimes to the point of almost complete disappearance in consequence of an autoclaved rice dietary. The same is true with regard to autoclaved food (Fig. 5). In one young monkey in the former category the thymus weighed 0.15 gram per kilo of original body-weight as compared with an average of 0.54 gram in health. In another baby monkey in the autoclaved food category, the thymus weighed but 0.08 gram per kilo of original body-weight. In most other cases the organ existed in traces only.

M. *The testicles.*—The degree of atrophy of the testicles observed in monkeys (Fig. 4) in consequence of the deficient dietaries is very much less marked than that found in pigeons. In the latter the testicles averaged in health as much as 4.8 grams per kilo of body-weight<sup>(2,3)</sup>, whereas in healthy monkeys they average but 0.257 gram per kilo. The atrophy of the testicles in monkeys was most marked in

FIGURE 4.

Showing the average weights of the adrenal glands, the testicles, the thyroid gland, and the pituitary body per kilo of *original* body-weight in monkeys fed on deficient dietaries :

Column A : Controls.

Column B : Autoclaved rice.

Column C : Autoclaved rice and butter.

Column D : Autoclaved food and onion.

Column E : Autoclaved food, onion and butter.

Note the enlargement of the adrenal glands in all four categories, and that of the pituitary in categories B, C and D; also the atrophy of the testicles in categories B, C and D and their freedom from atrophy in category E.

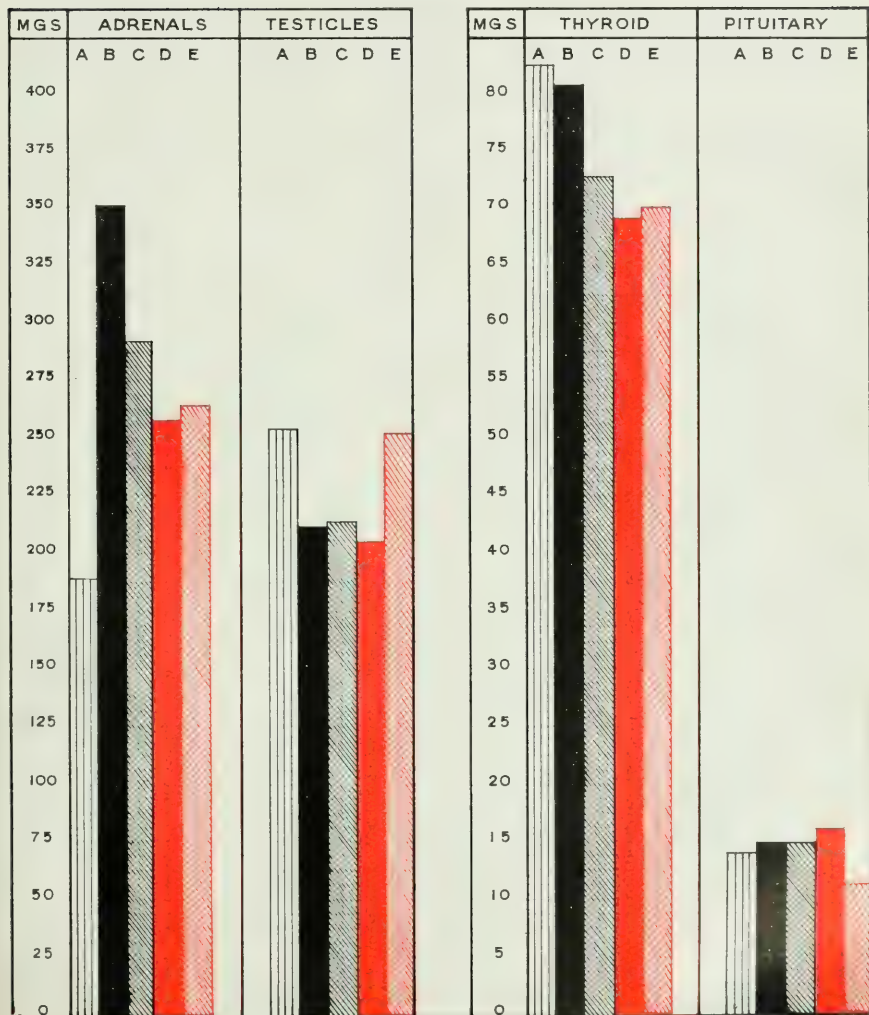






FIGURE 5.

Showing the average weights of the thymus, the submaxillary gland, the pancreas, and the spleen per kilo of *original* body-weight in monkeys fed on deficient dietsaries :

Column A : Controls.

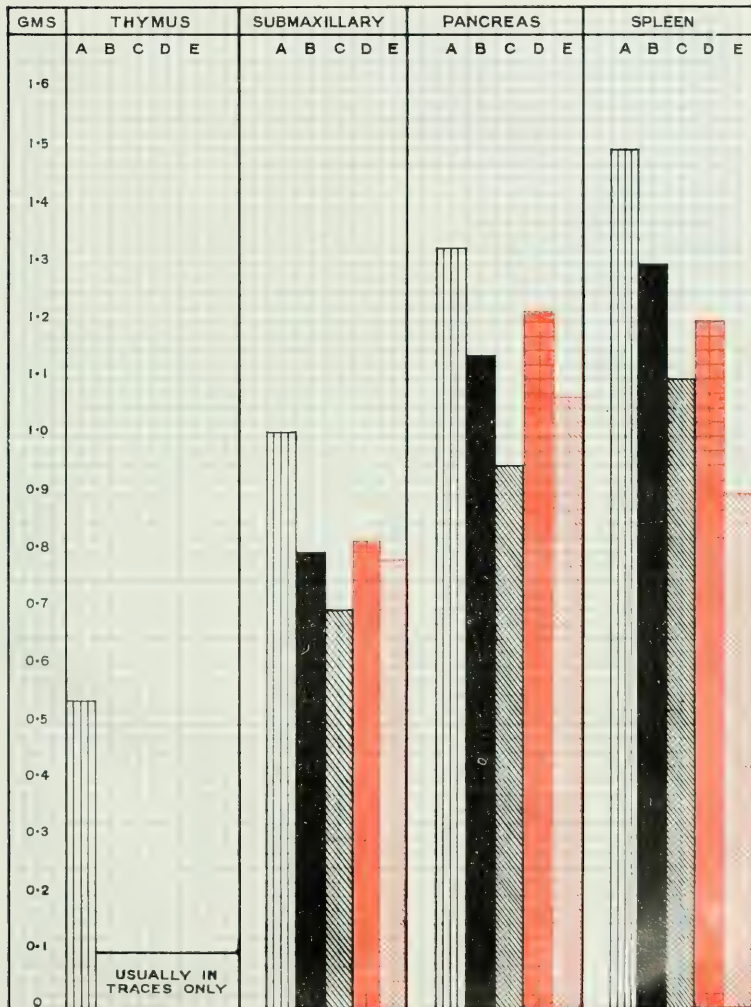
Column B : Autoclaved rice.

Column C : Autoclaved rice and butter.

Column D : Autoclaved food and onion.

Column E : Autoclaved food, butter and onion.

In all categories the thymus existed, as a rule, in traces only. Note the greater atrophy of the pancreas in butter-fed animals. Also the greater atrophy of the spleen in butter-fed animals.





those fed on autoclaved food with onions, that is to say, in those whose food was lacking in the 'A' as well as the 'B' factor. In animals from whose dietary the latter vitamine alone was wanting, the changes in the testicles, as indicated by weight, were trifling. Although the figures are too small to admit of positive conclusions, these results appear to indicate that some substance contained in butter exercises a notable influence on the nutrition of the testicles. In monkeys fed on autoclaved rice the atrophy of the testicles was, however, the same whether they received butter or not (Table III).

N. *The female reproductive organs.*—These organs were not weighed in monkeys. To the naked eye they appeared small and atrophic, the uterus being commonly flattened antero-posteriorly and having lost in great measure its plump, pear-shaped appearance. It was often congested under its serous covering, as also was the ovary. It will be remembered that during the late war 'amenorrhœa' was a frequent symptom of food deficiency.

O. *The spleen.*—The atrophy of the spleen which occurs in monkeys is not nearly so pronounced as that which occurs in pigeons (Fig. 5)(<sup>2,3</sup>). This is all the more remarkable since the weight of this organ per kilo of body-weight does not differ greatly in healthy pigeons and in healthy monkeys. In the former it was 1.48 grams per kilo in control pigeons receiving butter with their grains(<sup>2,3</sup>); in the latter it was 1.5 grams per kilo. Both the spleen and the testicles thus present in regard to their degree of atrophy a remarkable contrast in pigeons and in monkeys. The lesser atrophy of these organs in monkeys is one of the outstanding differences in the response of the two species to the action of food deficiencies. It is notable that the atrophy of the spleen is more considerable in butter-fed animals—a finding which has previously been recorded in the case of pigeons(<sup>3</sup>).

P. *The heart.*—It is a remarkable fact that in no case was hypertrophy of the heart, comparable to that found in human beri-beri, observed in monkeys. In all categories the atrophy of the heart was pronounced, being most marked in animals fed on autoclaved food and onions (Fig. 5). The weight of the heart in control animals ranged between 9.37 grams and 14.55 grams, with an average weight, per kilo of original body-weight, of 4.47 grams. In monkeys fed exclusively on autoclaved rice (adolescent animals) the range of weight was narrow, being from 5.25 grams to 7.3 grams, with an average weight, per kilo of original body-weight, of 3.53 grams. In those fed on autoclaved rice and butter (adult animals) the

weight of the heart ranged between 6.7 grams and 8.82 grams, with an average, per kilo of original body-weight, of 3.44 grams. In no case did the weight of the heart equal the minimum encountered in controls. Again, in animals fed on autoclaved food plus onion, the weight of the heart ranged between 3.27 and 4.5 grams in baby monkeys, and between 5.8 and 7.8 in adult animals: the average weight per kilo of original body-weight being for the six animals 3.03 grams. Only in monkeys fed on autoclaved food, butter, and onions did the weight of the heart equal in any case the minimum encountered in controls; indeed, no less than three were within the limits of health, being 9.65, 9.88, and 11.57 grams respectively. The remaining two, however, fell far short of the minimum of health, being but 7.4 and 7.5 grams respectively.

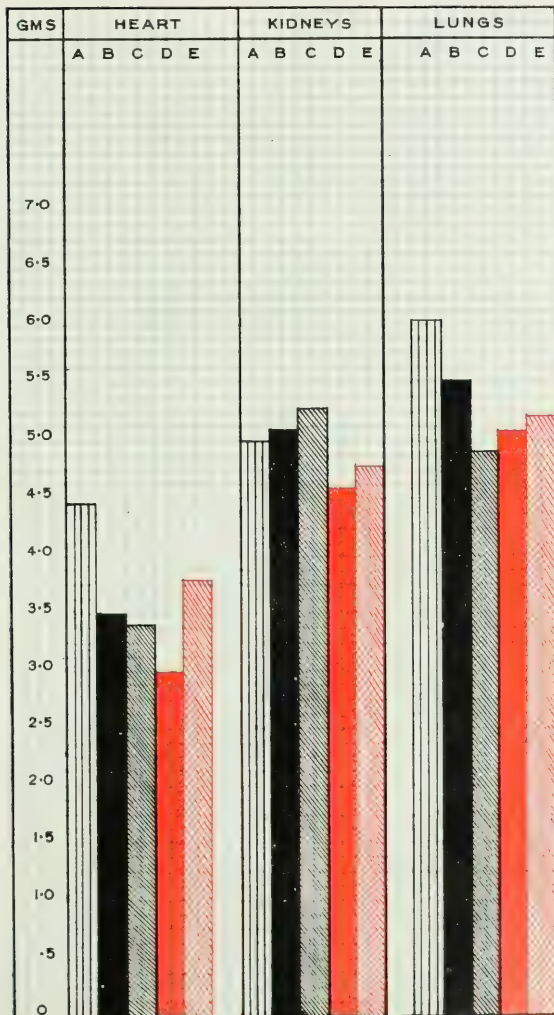
Nor was œdema of the auriculo-ventricular junction of the heart, so common in pigeons fed on autoclaved rice<sup>(2,3)</sup>, ever encountered in monkeys. Perhaps, the most notable finding with regard to the heart, apart from its atrophy in all categories, was the occurrence in two cases, fed on autoclaved food and onion, of ecchymoses under its serous surface. In one of these both auricles and ventricles were so affected, in the other the right ventricle only. The former case had four drops of fluid in the pericardium. This appearance was not found in animals other than those fed on autoclaved food and onion. Dilatation of the right heart was not infrequent.

Q. *The kidneys*.—The gravimetric findings with regard to the kidneys are peculiar (Fig. 6). In monkeys the basis of whose dietary was autoclaved rice, there was a slight tendency to increase in weight of these organs; in those, the basis of whose dietary was autoclaved food, there was a slight tendency to decrease in weight (Table III). The urine was examined at autopsy in a number of cases but with negative result in so far as the presence of albumen was concerned. The naked-eye appearances of the kidneys presented, as a rule, no noteworthy features: in some cases, however, the organs were noted to have been anæmic.

R. *The thyroids*.—In monkeys, as in pigeons<sup>(2,3)</sup>, the degree of atrophy of the thyroid is not great (Fig. 3). It is notable that the addition of butter to the dietary of autoclaved rice increased the degree of the atrophy; for whereas the average weight of the thyroid in monkeys fed exclusively on autoclaved rice approximated closely to that of health, being 0.081 gram as compared with 0.088 gram, in animals which received butter in addition to the rice it was but 0.073 gram. Attention may be directed to the fact that I have recorded a similar finding in pigeons<sup>(2,3)</sup>.

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Column A : Controls.  
Column B : Autoclaved rice.  
Column C : Autoclaved rice and butter.  
Column D : Autoclaved food and onion.  
Column E : Autoclaved food, butter and onion.









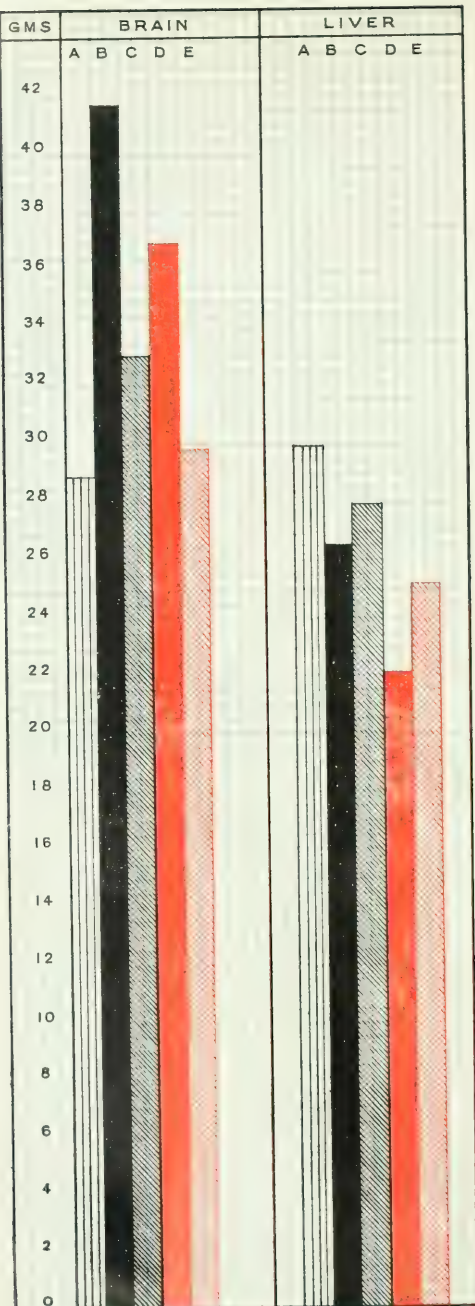


PLATE XLVIII.

FIGURE 7.

Showing the average weights of the brain and liver per kilo of original body-weight in monkeys fed on deficient dietaries :

Column A : Controls.

Column B : Autoclaved rice.

Column C : Autoclaved rice and butter.

Column D : Autoclaved food and onion.

Column E : Autoclaved food, butter and onion.

Note the greater weight of the brain in all four categories. Allowance has to be made for the fact that the animals were all adolescents in category B while half of them were adolescents in category D. Even with the correction for age the increased weight of the brain is a notable feature.

The considerable atrophy of the thyroid in monkeys fed on autoclaved food is noteworthy. It would be of great interest to ascertain by chemical methods of examination to what extent the secretion of the thyroid is influenced in its production and composition by the deficient dietaries. Histological and gravimetric methods of study are, in the case of these organs, but incomplete means of determining the degree of functional change.

S. *The lungs*.—The considerable loss of weight of the lungs, in all four categories, is noteworthy (Fig. 6), more especially as I found in pigeons fed on autoclaved rice, butter, and onions a marked increase in weight of the lungs<sup>(3)</sup>.

T. *The brain*.—With regard to no organ has the gravimetric method of study followed in these experiments yielded more remarkable results than in the case of the brain (Fig. 7). Its superficial vessels were often much engorged and in some animals, fed on autoclaved food, onion, and butter, a decided increase in cerebro-spinal fluid was observed. The weights of this organ in the four categories are not strictly comparable since the weight of the brain varies widely at different age periods in man and no doubt varies in like manner in monkeys. The controls are, however, comparable with animals fed on autoclaved rice and butter, and with those fed on autoclaved food, butter, and onion. It will be noted that in the last two categories the average weight of the brain per kilo of original body-weight is considerably higher than in controls, being markedly so when the dietary is excessively rich in starch as well as in butter (Fig. 7)—a finding which confirms that previously recorded in pigeons<sup>(3)</sup>. In control pigeons the weight of the brain per kilo of body-weight was 6.262 grams, while in those fed on autoclaved rice, butter, and onion it was 7.212 grams per kilo of original body-weight, or about one-seventh part heavier than in health. In monkeys the corresponding weights are for health 28.8 grams, and for the diseased state 32.4 grams, or again about one-seventh part heavier than in health. It appears to be more than a coincidence that in both species the addition of butter to an autoclaved rice dietary should cause an increase in weight of the brain of one-seventh its normal weight. It may be taken then as established that the weight of the brain is markedly increased by a dietary excessively rich in starch and fats and deficient in proteins and accessory factors of the 'B' class. These findings indicate that the brain is capable of considerable variations in weight, and it may be presumed also of size dependent upon the composition of the food. The importance of this

observation with respect to the occurrence of headaches, mental confusion, lack of concentration, and other evidences of mental disorder in man would appear to be considerable. The influence of diet on the causation of mental disease has been emphasized by Dr. Charles Mercier<sup>(11)</sup> as a result of his clinical observations and lucid reasoning. The observations here recorded appear to provide the experimental proof of his contentions: he found that headache was a frequent consequence of a dietary excessively rich in fats and starch and that deficiency of meat was a potent cause of mental confusion. A rate of between 94 to 95 per cent of recovery, or very great improvement, occurred in his practice amongst those exhibiting these evidences of mental disorder whose dietary was corrected.

It seems then that the weight of the brain is largely a question of the quality of the food and that when protein and accessory food factors of the 'B' class are wanting and starch and fats are in excess the brain-weight, and presumably also its bulk, is increased. This change is attributable to alterations in the composition of the blood. That pronounced changes in the brain volume can be produced by alterations in the osmotic pressure of the blood has recently been demonstrated by Weed and McKibben<sup>(10)</sup>. Such alterations were brought about by these observers by the intravenous injection of hypotonic and hypertonic solutions. Amongst the factors in the deficient dietaries, employed in these experiments, which are likely to bring about such alterations in osmotic pressure, lack of protein is one: but I have found that while the weight of the brain increased markedly in pigeons fed on autoclaved rice, butter, and onion, no such increase occurred in pigeons fed exclusively on autoclaved rice, on a diet, in short, which is poorer in protein than the autoclaved rice-butter-onion dietary. It would seem then that it is the disproportionate presence of fats, in association with the absence of 'B vitamine,' and not the insufficient supply of protein, which is responsible for the increase in weight of the brain—a conclusion which is borne out by the increased weight of the organ in monkeys fed on autoclaved food, butter, and onions. It is questionable, however, whether an excess of fat in the dietary would in itself give rise to an increase in weight of the brain without the intervention of a deficiency of accessory food factors of the 'B' class. The practical application of this finding providing as it does confirmation of Dr. Charles Mercier's clinical studies<sup>(11)</sup>, is the necessity for adjusting the vitaminic, protein, starch, fat, and salt balance of the dietary in every case of headache, or mental disorder.



PLATE XLIX.

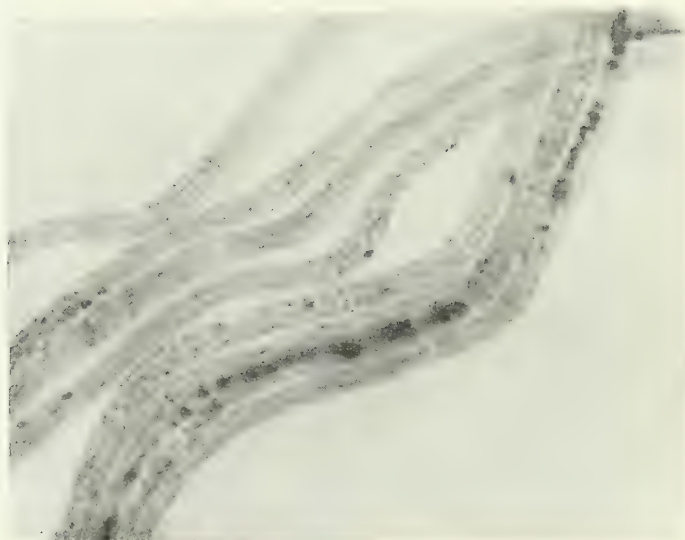


Fig. 8. Fibres of the femoral nerve showing typical Wallerian degeneration. From a monkey fed on autoclaved food and onion,  $\times 250$ .

U. *The nerves.*—For diagnostic purposes portions of the femoral nerve or of its branches in the thigh were removed in all 33 animals. These were treated by Marchi and Algeri's method for the demonstration of fatty degeneration in the myelin-sheath. The familiar appearances of Wallerian degeneration (Fig. 8) were taken as the criterion of degenerative change. In addition to these appearances the staining of the myelin-sheath of some fibres was of a more diffuse and patchy character. As, however, such appearances are capable of being produced in nerves from apparently healthy animals, in consequence, it may be, of stretching at the time of removal, no nerve was classed as degenerated unless it contained some fibres exhibiting the characteristic Wallerian appearances. Minute precautions were taken at the time of autopsy to avoid injury to the portions of nerve removed for examination. While, therefore, my estimate of the degree of degenerative change attributable to the various deficient dietaries may be too low, it is certainly not too high. The incidence of nerve fibre degeneration in the peripheral nerves of the 33 animals was as follows: Controls, *nil*; autoclaved rice, two; autoclaved rice and butter, one; autoclaved food and onion, four; autoclaved food, butter, and onion, two. It would seem from these results that the incidence of nerve fibre degeneration is largely a question of length of exposure to the dietetic deficiency. While the high percentage of cases exhibiting degenerative changes amongst animals fed on autoclaved food was, no doubt, due to deficiency of 'B-vitamine,' it is to be noted that animals receiving butter in addition to autoclaved food showed a lesser incidence of nerve fibre degeneration than those receiving autoclaved food and onion only. The number of degenerated fibres varied in each case within fairly wide limits. In monkey No. 29 fed on autoclaved rice and butter two fibres only were encountered which presented typical Wallerian degeneration. In other cases the proportion was considerably higher: a rough estimate of the number of fibres involved being about 5 to 10 per cent. These findings indicate that the weakness of the limbs, referred to in the section dealing with clinical observations, is to be attributed, in part at least, to degenerative changes in the nervous system. The Wallerian character of the degeneration (Fig. 8) would appear to point to degenerative change in certain cells of the central nervous system.

V. *The effects of an excess of butter in ill-balanced dietaries.*

It will have been noted that the addition of butter to a dietary of autoclaved rice, that is to say, to one deficient in vitamins and protein

while at the same time excessively rich in starch, gives rise to some remarkable results :

(1) Animals so fed died earlier and lost weight more rapidly than those fed exclusively on autoclaved rice. A similar result was also observed in pigeons in certain experiments<sup>(3)</sup>.

(2) Fatty acid crystals were present in excess in their motions.

(3) The brain-weight was considerably greater in monkeys to whose deficient dietary butter was added, and notably greater than in health ; a finding which applies alike to pigeons<sup>(3)</sup> and monkeys.

(4) A greater degree of atrophy of the pancreas occurred ; a finding which applies alike to pigeons<sup>(3)</sup> and monkeys.

(5) A greater degree of atrophy of the thyroid occurred ; a finding which applies alike to pigeons<sup>(3)</sup> and monkeys.

(6) The heart also tended to atrophy more ; a finding which applies alike to pigeons<sup>(3)</sup> and monkeys.

(7) The lungs atrophied more, whereas in butter-fed pigeons<sup>(3)</sup> the weight of these organs increased.

(8) The spleen atrophied more ; a finding which applies alike to pigeons<sup>(3)</sup> and monkeys.

(9) The submaxillary glands atrophied more.

(10) The gastro-intestinal tract had imparted to it a yellow-white tinge.

Changes so remarkable as these are not without an important significance.

#### VI. SUMMARY OF RESULTS.

1. Monkeys fed exclusively on rice, autoclaved at a temperature of 130°C. for 1½ hours, died in an average period of 23·4 days.

2. Monkeys fed on rice, similarly autoclaved, to which fresh butter was added died in an average period of 15 days. The addition of butter to the dietary (excessively rich in starch and deficient in vitamins and protein) hastened the death of animals. A similar finding has previously been recorded in the case of pigeons<sup>(3)</sup>.

3. Monkeys the basis of whose dietary was autoclaved food survived much longer than those the basis of whose dietary was autoclaved rice : an average 70 days as compared with an average of 20 days. The more liberal provision of protein and the more perfect balance of the food with respect to protein and carbohydrates prolonged the life of the animals in the former category.



4. Monkeys (*macacus sinicus*) cannot sustain life for periods much longer than 100 days on a dietary which is devoid of accessory food factors of the 'B' class. Lack of this factor is a fundamental cause of the animals' dissolution; deficiency of protein, excess of starch, and excess of fat in the absence of 'B-vitamine' contribute to this dissolution.

5. The total loss of weight in monkeys of all four categories was from 25-32 per cent of the original weight of the animals; the greatest loss being in those fed on autoclaved food and onion, that is, in those which survived the longest time. Those to whose dietary of autoclaved rice butter was added, lost weight more rapidly than those receiving no butter.

6. The chief clinical evidences of disease due to the deficient dietaries were progressive anæmia and asthenia, loss of appetite, diarrhoea, dysentery, diminished sensibility, weakness of the limbs, headache, impaired nutrition of the skin, subnormal temperature, and enfeebled heart's action. Symptoms referable to the nervous system were less prominent and appeared later than those referable to the digestive system; they were for the most part obscured by the profound asthenia.

7. The symptoms were similar in all four categories, but manifested themselves later in monkeys whose food was more perfectly balanced with respect to protein and carbohydrates.

8. Symptoms referable to the digestive system were on the whole less common in monkeys whose food was more perfectly balanced with respect to protein and carbohydrates. Nevertheless gastritis and colitis were frequent clinical features in these animals.

9. Dropsy was not present in any of the monkeys fed on the deficient dietaries.

10. Hæmic infections, as determined by aerobic culture of the heart's blood at autopsy, were commonly present in monkeys whose food was excessively rich in starch and deficient in protein and vitamins.

11. All classes of deficient dietaries caused in monkeys an increase in weight of the adrenal glands. This increase was more marked in those fed exclusively on autoclaved rice and was associated with an increase in the adrenalin-content of the glands, provided no hæmic infection was present. No increase in the adrenalin-content of the suprarenal glands occurred in monkeys to whose dietary of autoclaved rice butter

was added. Similar findings have previously been recorded in pigeons.

No increase in the adrenalin-content of the suprarenal glands was found in monkeys whose deficient dietaries were more perfectly balanced with respect to proteins and carbohydrates. The adrenalin-content of the suprarenal glands was below the average of health in monkeys presenting hæmic infections.

12. The left adrenal was one-seventh part heavier than the right per kilo of body-weight in healthy monkeys. This proportion was maintained when the animals were fed on autoclaved rice or on autoclaved food. When, however, butter was added to the autoclaved rice or to the autoclaved food the difference in weight between the left and the right adrenal was more marked; the left adrenal was then one-fourth to one-fifth part heavier than the right.

13. The deficient dietaries gave rise in all categories to an increase in weight of the brain. When similarity in the age of the monkeys rendered the findings in the different categories comparable it was noted that the increase in weight of the brain was more marked in those whose food was excessively rich in butter and starch and at the same time deficient in protein and vitamin. A similar finding has previously been recorded in pigeons. The increase in weight of the brain in these circumstances amounts in monkeys to approximately one-seventh part of its normal average weight per kilo of original body-weight; it amounts also in pigeons to approximately one-seventh part of the normal average per kilo of original body-weight. It is suggested that this finding may account for the headache and other symptoms observed in certain cases of mental disorder in the human subject.

14. Three classes of deficient dietaries, *viz.*, (a) autoclaved rice, (b) autoclaved rice and butter, and (c) autoclaved food and onion, caused in monkeys an increase in the weight of the pituitary body. This organ was found to be decreased in weight in monkeys fed on autoclaved food, butter, and onion. Similar findings have been recorded in the case of pigeons fed on the first two classes of deficient dietaries above-mentioned.

The pituitary gland was heavier per kilo of body-weight in female than in male control monkeys. A similar finding has been recorded in the case of pigeons. The pituitary gland increased in weight in male but not in female monkeys in consequence of an exclusive diet of autoclaved rice; a similar finding has been recorded in the case of pigeons.

15. Atrophy of all other organs—the thyroid, the reproductive organs, the thymus, the submaxillary gland, the pancreas, the spleen, the liver, the heart, the kidneys, the lungs—occurred in consequence of the deficient dietaries. The kidneys, however, showed a diminution in weight only in those animals the basis of whose dietary was autoclaved food. The atrophy of the testicle also was slight in monkeys fed on autoclaved food, butter, and onion.

16. The atrophy of the pancreas, the thyroid, the spleen, and the heart was more marked in monkeys to whose deficient dietary of autoclaved rice butter was added. A similar finding has previously been reported in pigeons. The atrophy of the submaxillary gland was likewise more marked in monkeys whose deficient dietaries were excessively rich in butter.

17. The decrease in weight of the liver was not so marked in monkeys to whose dietary of autoclaved rice or of autoclaved food butter was added. A similar finding has been recorded in the case of pigeons fed on autoclaved rice, butter, and onion.

18. In addition to the enlargement or atrophy of the organs above referred to, the main pathological states observed in monkeys at autopsy were dilatation of the stomach, gastritis, duodenitis, enteritis, ballooning of the small intestine, intussusception, colitis, atrophy of the muscular coats of the bowel, complete loss of fat from the omentum, and enlargement of the abdominal lymphatic glands.

19. The excessive starch element, in the absence of protein and vitamins, appeared to be largely responsible for the great dilatation of the stomach, the ballooning of the bowel and the 'air-locks' in the intestine. The stomach and the small bowel were less affected in monkeys whose food was more perfectly balanced with respect to protein and carbohydrates. In general the gastro-intestinal tract was not so frequently nor so extensively diseased in animals fed on autoclaved food, butter, and onion, although both gastritis and colitis were present in 40 per cent of these animals.

20. In all categories atrophy of the heart occurred; it was less marked in animals fed on autoclaved food, butter and onion. In no case was hypertrophy of the heart encountered. Slight hydropericardium occurred in a small percentage of cases.

21. The reproductive organs atrophied to a considerable extent in both sexes, but the atrophy was much less marked than that previously

found in pigeons in like circumstances. Sub-serous congestion of the uterus and ovaries was frequently present.

22. The atrophy of the spleen was less marked in monkeys than in pigeons similarly fed.

23. The weight of the lungs decreased in monkeys in all categories, thus contrasting markedly with pigeons in which an increase in weight of these organs was noted to result from a dietary of autoclaved rice, butter, and onion.

24. Post-mortem evidences of œdema were very scanty in all four categories.

25. Degenerative changes were present in the femoral nerves as follows : Controls, *nil* ; autoclaved rice, two ; autoclaved rice and butter, one ; autoclaved food and onion, four ; autoclaved food, butter, and onion, two.

#### CONCLUSIONS.

1. Dietaries which are deficient in vitamins and in protein, and at the same time excessively rich in starch or in fat or in both, are potent sources of disease and especially of gastro-intestinal disease.

2. An excess of fat, in association with deficiency of 'B-vitamine' and protein and superabundance of starch, is peculiarly harmful to the organism.

3. Certain dietetic deficiencies greatly favour the invasion of the blood and tissues by bacteria, especially is this the case when deficiency of vitamins and protein is associated with an excessive intake of starch.

4. Since life cannot be sustained in *macacus sinicus* for much longer than 100 days on a dietary almost wholly devoid of 'B-vitamine,' it would appear that complete absence of this vitamine from the food of human beings is of less practical importance, from the point of view of disease production, than is its subminimal supply. Complete deprivation of 'B-vitamine,' especially in the presence of imperfect balance in other essential requisites of the food, will lead to rapid dissolution and death ; subminimal supply of this vitamine will lead, in like circumstances, to slow dissolution and disease.

5. It is thought that the findings recorded in this paper may afford some explanation of the genesis of that great mass of ill-defined gastro-intestinal disorder and vague ill-health which forms so high a proportion of human ailments at the present day.

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# THE PATHOGENESIS OF DEFICIENCY DISEASE.

## NO. IX. ON THE OCCURRENCE OF RECENTLY DEVELOPED CANCER OF THE STOMACH IN A MONKEY FED ON FOOD DEFICIENT IN VITAMINES.

BY

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IN the present issue of this Journal I have recorded the effects on monkeys of certain deficient dietaries. The occurrence of a recently developed cancer of the pylorus in one of these animals may have been a fortuitous circumstance unconnected with the dietetic defect. On the other hand, it is possible that the malignant growth may have been connected in its origin with the deficiency of certain food factors; in this event the observation would assume a high importance.

The facts of the case are these: The animal was an adult female monkey of the species *macacus sinicus*. It was captured in the local jungles some few days prior to its arrival in my laboratory. It was very vigorous and appeared to be in perfect health. It weighed 2,400 grams. It was fed for the first ten days of the experiment on rice and butter—120 grams of the former and 15 grams of the latter. The rice was autoclaved at a temperature of 130°C. for one hour-and-a-half. Of this food the animal ate freely at first, more sparingly towards the end of the ten days. On the eleventh day the diet was changed to 60 grams of rice, 60 grams of wheaten bread, 120 grams of milk, 10 grams of ground-nuts, 15 grams of fresh butter, and 5 grams of onion. The rice, bread, milk, and ground-nuts were autoclaved at a temperature of 130°C. for one hour-and-a-half in order to destroy completely their content of B-vitamine. The butter and onions were given fresh. The food thus

contained an adequate supply of 'A' and 'C' vitamins but was deficient in 'B' vitamin. The addition of butter and onions to a dietary of autoclaved rice does not prevent the onset of 'polyneuritis' in pigeons nor the ultimate death of the birds. Consequently the 15 grams of butter and the 5 grams of onions included in the monkey's ration did not contain sufficient B-vitamin to maintain the healthy function of the tissue cells.

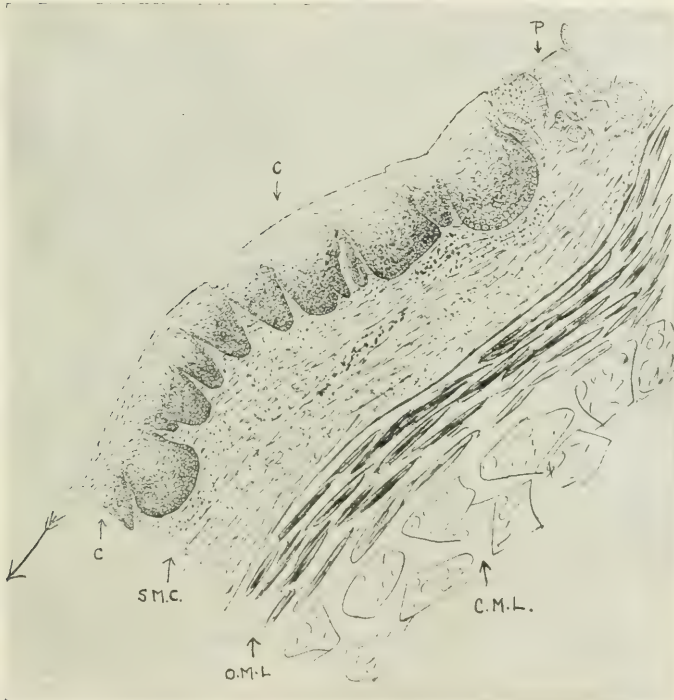


FIG. 1.—Semi-diagrammatic drawing of section from pyloric end of stomach of a monkey fed on autoclaved food, butter, and onion, showing C, carcinoma; P, pyloric glands; S.M.C., submucous coat; O.M.L., oblique muscular layer; C.M.L., circular muscular layer. Drawing represents one complete field under Zeiss objective AA and ocular No. 4. The growth extended for an additional two-and-a-half fields of the microscope in the direction of the large arrow.



The animal survived this dietetic regime for 51 days. Its weight at death was 1,950 grams, a loss of 450 grams. It exhibited during the course of the experiment the following symptoms: diarrhoea, progressive anæmia, asthenia, and, during the last nine days, severe dysentery. The animal was autopsied immediately after death.

The stomach was greatly dilated but presented no other naked-eye evidences of disease. Following my laboratory routine in such cases, a portion of the stomach, one centimetre square, embracing all coats of the organ, was removed from the neighbourhood of the pylorus for sectioning. The tissue was fixed in Zenker's fluid, embedded in paraffin, sectioned and stained with hæmatoxylin. Three such specimens were examined. In one the appearances seen under a low power of the microscope (Zeiss Obj. AA. Oc. 4) are represented semi-diagrammatically in the drawing (Fig. 1). An obvious carcinoma was present. It was of small area, covering in a transverse direction  $3\frac{1}{2}$  fields of this power of the microscope. The surface of the growth was flush with the mucous surface of the stomach and exhibited no excrescences towards the cavity of the viscus. Numerous epithelial down-growths (accurately represented in the drawing) projected into the submucous coat. This coat appeared somewhat thickened beneath the growth. The down-growths had not penetrated deeply into the submucosa; the vertical area occupied by them scarcely exceeded that which would have been occupied by the glandular elements they replaced. The cells near the advancing edge of the growth stained well, their staining capacity gradually diminishing towards the mucous surface of the stomach. The older cells were of hyaline appearance, their nuclei having disappeared or having failed to retain the stain (Fig. 1). The two remaining specimens were not in series with the first; they showed but occasional microscopic nodules of the carcinoma, one of which is shown in the photo-micrograph (Fig. 2). Further sections taken from the same block showed no growth.

The discovery of this area of carcinoma was largely a matter of chance since there was no naked-eye evidence of its presence. Such early cancers may very easily be missed unless microscopical search is made for them throughout serial sections of the organ. It may be that this carcinoma was the only case of cancer of the stomach in my series of monkeys; on the other hand it is quite possible that I may have missed others. That the carcinoma was of recent origin seems probable since the area of pyloric mucosa involved by it was of very small size. Its superficial area in a transverse direction did not, in the sections examined,

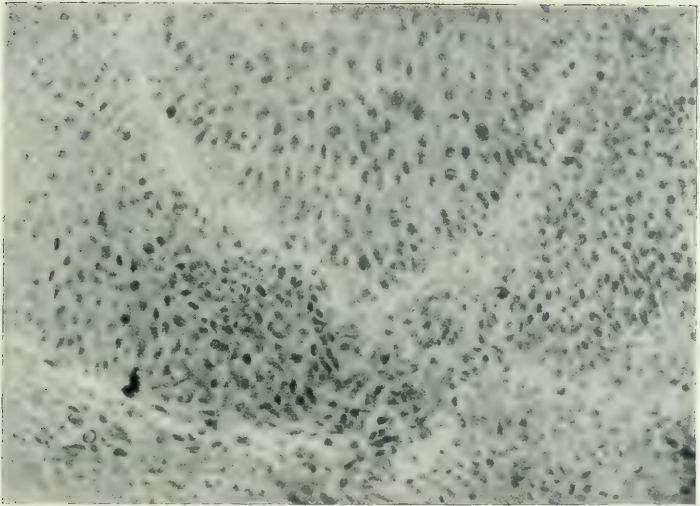


Fig. 2.—Microphotograph,  $\times 165$ , of recently developed carcinoma of the pylorus in a monkey fed for 51 days on food almost wholly devoid of B-vitamine.



exceed  $3\frac{1}{2}$  fields of the low power of the microscope. I have no evidence as to its extent in a longitudinal direction. The growth barely extended in point of depth beyond the limits of the mucous membrane, its invasion of the submucous coat being but slight. The deeper coats of the stomach were unaffected and no puckering or contraction of the walls of the viscus had occurred. For these reasons I am inclined to think that the carcinoma was of recent origin and that in all probability it developed during the 51 days of the experiment. However this may be, the experience would seem to justify the prosecution of an enquiry into the possible influence of vitaminic deficiency in favouring the onset of cancer of the stomach.

# THE TINTUROMETER, AN INSTRUMENT FOR MEASURING TINT AND TURBIDITY.

BY

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THE word used to designate the instrument which I am about to describe is intended to compound the ideas of measurement of tint and turbidity in one word. It is not too cumbersome for common use. It is a hybrid word in its derivation, but convenience and clearness of description may well serve to override this objection to its use. The utility of having an instrument which will serve to measure the concentration of suspensions whether tinted or colourless and of measuring the depth of tint of a solution is sufficiently obvious, if the claim to its capability of doing so can be substantiated. The instrument which I have devised has been used to measure (1) the degree of growth of organisms in fluid media of different composition, (2) the concentration of erythrocytes in the blood, and (3) the hæmoglobin content of the blood. In the case of the growth of organisms in media of different composition, a difficulty arises in that we may have, to some extent, to measure degrees of turbidity which are complicated by the fact that various media may have somewhat different tints. The difficulty can be got over, in so far as the effect of differing tint is a significant one, by measuring the effect of the colour of the medium itself, apart from the growth which has taken place in it and which has given rise to turbidity. In the case of the measurement of the erythrocyte content of the blood there are at least two complicating factors—the variation in hæmoglobin independently of erythrocytes and the effect on turbidity which is due to leucocytes. But the degree of concentration of hæmoglobin for total volume can be measured separately, as can be done in the case of tint of a medium.

The measurement of hæmoglobin content can then be compared with the normal and so an estimate formed of the degree of turbidity as a measure of purely erythrocyte content; for the use of this instrument, as in the case of most such instruments, involves the use of a standard of comparison—a fixed standard, or the standard which may be regarded as 'normal.' The measurements necessary to establish these standards are made with the instrument and are to be initially determined. The standard or 'normal' can easily be measured, if desirable, on each separate occasion that the instrument is used. This may even be absolutely necessary if conditions of lighting and other conditions of observation are not capable of being initially and finally fixed. The objection that the method described of measuring erythrocyte content of the blood takes no account of leucocyte content as a disturbing factor, is the same objection which is constantly raised to the use of the hæmatocrit in the measurement of blood corpuscular volume. It may have some cogency in certain very abnormal states of the blood, but in the large majority of measurements the effect is not likely to be significant.

1. *Description of apparatus.*—It is simple in the extreme and consists only of a cylindrical measure glass and a steel foot rule. The cylinder measure which I have used is one of 10 c.c., chosen so that its foot was free from bubbles included in the glass. The 10 c.c. is contained in a height of about 12 cm., and is subdivided in graduations equal to 0.2 c.c. The steel rule is simply a convenient means of supplying a figure whose degree of visibility is to be the measure sought for. It is obvious that a special instrument could be manufactured for the purpose of these measurements, with a specially thin foot and with graduations in one-tenth instead of two-tenth cubic centimetres. It could also have certain standards prepared and sent out with the instrument. There is disadvantage and fallacy in the use of this instrument with light which passes down to the standard figure or type employed and then passes up the column again to the eye. The light is in this way subjected to a double and not a single passage through the test fluid, with the result that a much greater differential effect will be produced by thick suspensions or by depth of colour than by thin suspensions or light colouration. The disadvantage can be got over by surrounding the cylindrical portion of the instrument with a movable outer covering of black paper or cardboard, when the light which traverses the suspension will only traverse it in an upward direction. Better still would be an arrangement to provide substage illumination from below the foot of the instrument. A transparent celluloid plate inscribed with a number could take the place of the steel rule. Either of these arrangements would ensure or could be made to ensure that only approximately parallel rays traverse

the column of fluid. If this condition be observed, then it is reasonable to suppose that the amount of light absorbed—that is, the decrease in intensity—at any level depends upon the intensity of light which reaches that level and upon the concentration of light absorbing particles.

2. *Method of use.*—(1) Fill the cylinder measure with the fluid to be tested. (2) Place the foot rule over a sheet of white paper in front of a window. (3) Stand the cylinder over a selected number<sup>(1)</sup> on the foot rule. (4) Remove the fluid from the cylinder, by means of a capillary pipette furnished with a teat, until the selected number just becomes visible<sup>(2)</sup>. (5) Read off the number of cubic centimetres left in the cylinder. (6) Compare this number with that given by the normal or standard fluid<sup>(3)</sup>.

*Notes.*—<sup>1</sup> It is advisable always to use the same number, as the eye becomes accustomed to its appearance. Any type of printing will also serve the purposes of this measurement instead of a number on a steel rule, provided the same print is always used.

<sup>2</sup> Practice will establish a standard limit for visibility, which, other things being equal, may then be the measure of light absorptive power of the test suspension or solution. It does not matter that the standard may differ slightly in different hands. Each individual establishes his own standard. Until such a standard is so established, or if the conditions of lighting are very variable, it will be necessary to employ a normal or standard suspension or solution along with the test fluid on each occasion of use. Instead of subtracting fluid until the selected figure becomes just visible, the instrument may be used, if desired, in the reverse way—the test fluid may be added to that in the cylinder until the number just disappears from sight.

<sup>3</sup> The absolute numbers themselves, without being represented as a fraction of the standard or normal, are sufficient for purposes of inter-comparison if the same conditions prevail throughout the experiment.

Supposing that the original colour of the medium and its light absorptive effect has to be considered apart from that of the particles which may be in suspension, the method has to be slightly modified. The effect of the colouration by itself alone has to be measured. Thus, if we are comparing the growth in two fluids of different colour or of different degrees of colour, we state the relationship of the growth in the one to that in the other as equal to an expression in which the figure of absorption for the one fluid without growth, divided by the figure for the other without growth, is multiplied by the difference between the figures for growth and non-growth of the first fluid, divided by the difference between the figures for growth and non-growth of the second fluid. This complicated description of the method of reading results—which is dependent for its validity on the truth of a law called Beers law—is expressed much more simply in formula form. If Y1 is the figure for absorption as given by the tinturometer for fluid A before growth has taken place in it, Y2 that for fluid B before growth has taken place, Y3 that for fluid A after growth has taken place in it, and Y4 that for fluid B after growth has taken place in it, then—

$$\begin{aligned} \text{Growth in A} &= \frac{Y1}{Y2} \times \frac{Y1-Y3}{Y2-Y4}. \end{aligned}$$

To take an example :—

Say that the tinturometer figure for fluid A was 9 before growth and 1.5 after growth and that the figure for fluid B was 6 before growth and 0.5 after growth, then—

$$\frac{\text{Growth in A}}{\text{Growth in B}} = \frac{9}{6} \times \frac{9-1.5}{6-0.5} = \frac{9}{6} \times \frac{7.5}{5.5} = 0.2.$$



3. *Standards*.—Permanent standards may be set up, if sufficiently stable and if comparable suspensions can be found for the purpose. As a simple turbidity standard for uncoloured suspensions, a 1-8 dilution of 1 per cent barium sulphate in 1 per cent sodium citrate serves the purpose excellently for measurement of turbidity due to bacteria in suspension. The mode of preparation of such a suspension has been described by Brown and Kirwan (1915), and by Brown (1919), and the correlation of opacities of graded suspensions made on this basis given with number and weight of organisms in tabular form. Coloured suspensions and coloured solutions can be similarly used or these standards may be made up at the time of use of the instrument.

4. *Uses*.—The possible uses of the instrument are very numerous. They range over all methods for the measurement of turbidity and of colorimetry. The original reason for devising the instrument was to find one which should be capable of measuring bacterial growth suspensions of greater tenuity than were capable of being quickly measured otherwise. It has been applied to the measurement of corpuscular content and hæmoglobin content of blood. It may be used for the standardization of vaccines, for the colorimetric measurement of hydrogen ion concentration of nutrient media, for the quantitative estimation of albumin or sugar in the urine, for the measurement of tryptic or anti-tryptic power and for many other purposes.

5. *Advantages*.—The great advantage of the instrument is its immediate readiness for use and for repeated use without time-consuming preparation. It is easily and quickly cleaned, and so very large numbers of observations can be made in a short space of time. The whole operation of estimation of result takes so little time that very little additional labour is involved in setting up standards and controls for repetition of measurement at each time of use, if so desired. The estimation required is accompanied with very little eye-strain and so possesses an advantage over any microscopical method. The method possesses a very great range of use because of its applicability to very dilute suspensions. Concentrated suspensions can be diluted before measurement. One great advantage, and that no small one, is that dilutions of the test fluid can be made in the measure cylinder itself as in the case of Example C.

6. *Examples of use*.—A. Standardization of the Instrument. A 0.1 per cent suspension of finely precipitated barium sulphate in 1 per cent sodium citrate is used for standardization. The following

results were obtained with varying dilutions of the barium sulphate suspension.

TABLE I.

Dilution.						Tinturometer reading.
0	.	.	.	.	.	0.4
2-fold	.	.	.	.	.	0.7
4-fold	.	.	.	.	.	1.5
8-fold	.	.	.	.	.	2.6
16-fold	.	.	.	.	.	3.8
32-fold	.	.	.	.	.	Over 10

B. Degree of Sensitiveness of the Instrument—for the reading of bacterial concentration. A 24-hour broth culture of *B. typhosus* was taken and diluted: 9 parts culture to 1 part diluent, 8 parts culture to 2 parts diluent, and so on. The readings of each of these dilutions were taken as well as that of the undiluted culture. The results are given of two separate trials of different 24-hour growths in the following table.

TABLE II.

Dilution culture.						TINTUROMETER READING.	
						I.	II.
10-10	.	.	.	.	.	0.7	0.8
9-10	.	.	.	.	.	0.8	0.8
8-10	.	.	.	.	.	0.9	0.9
7-10	.	.	.	.	.	1.0	—
6-10	.	.	.	.	.	1.1	—
5-10	.	.	.	.	.	1.2	1.3
4-10	.	.	.	.	.	1.3	1.4
3-10	.	.	.	.	.	1.6	1.5
2-10	.	.	.	.	.	—	2.2
1-10	.	.	.	.	.	—	3.4

C. A dilution of human blood 1-2000 was made with citrated salt solution. The measurement in the tinturometer of this dilution was given by the figure 2.2. This dilution was again diluted by taking 8 volumes and adding to it in the tinturometer one further volume of diluent. The tinturometer was easily able to differentiate between these dilutions of blood, and so should be able to differentiate at least between a corpuscular content of 5,000,000 per c.mm. and 4,000,000 per c.mm. The probabilities are that it would distinguish much slighter differences

of concentration than this. With blood, manipulations for removal of suspension to obtain standard visibility have to be very rapid, as the erythrocytes are heavy and settle rapidly. This difficulty is not present in the case of bacterial suspensions.

D. Degree of Sensitiveness of the Instrument—for the reading of colour intensity. A 1-10 dilution of blood was made with N-10 hydrochloric acid and this was again diluted 1-5 with the same diluent. The result was a clear solution of acid hæmatin. This solution was diluted as in the case of the bacterial suspension—9 parts hæmatin solution to 1 part distilled water, 8 parts solution to 2 parts distilled water, and so on. The results of two trials on the same solution were :—

TABLE III.

Dilution of hæmatin solution.	TINTUROMETER READING.	
	I.	II.
10-10 . . . . .	0·7	0·7
9-10 . . . . .	0·7	0·7
8-10 . . . . .	0·8	0·8
7-10 . . . . .	0·9	0·9
6-10 . . . . .	1·0	1·0
5-10 . . . . .	1·2	1·2
4-10 . . . . .	1·4	1·4
3-10 . . . . .	1·6	1·7
2-10 . . . . .	2·4	2·4
1-10 . . . . .	3·3	3·4

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## MEASUREMENT OF BACTERIAL CONTENT IN FLUID SUSPENSION.

BY

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THE measurement of bacterial content in suspensions is a very common operation in bacteriology. It is required for the standardization of vaccines and of suspensions of bacteria in serological tests. The standardization may take the form of counting of bacteria, counting the colonies to which they give rise, weighing, or estimation by turbidity produced. In these studies I have used the tintometer, an instrument for measuring the tint or turbidity of fluids, and which is described in the present number of this journal. This instrument consists of a graduated cylinder measure with a foot which is free from air bubble flaws. This cylinder, which contains the fluid to be measured, is placed over standard print and the print read through the column of fluid. The point of liminal visibility is taken as the measure of the turbidity of the suspension under test and the quantity of fluid remaining in the cylinder when this point is reached is the figure used in comparison. The smaller this figure, the more turbid or the more concentrated is the fluid under test. An opaque sliding sheath over the cylinder prevents the entrance of rays of light which would pass downwards through the suspension itself before being reflected upwards again through the column to the eye. These points have all been considered and discussed in the paper to which I have referred. Experience very quickly establishes for the individual the liminal point of visibility. This will vary until judgment has been formed as to the most convenient point to take. In the earlier experiments which I conducted with this instrument the point chosen was one of minimal visibility with strained accommodation. Later, this was

changed to one of comfortable visibility without strain of accommodation. In both cases the point was the liminal one for the conditions chosen, but the earlier experiments set out in this paper are not—owing to the change of conditions—exactly comparable with the later. This is not material to the conclusions drawn from each experiment, as each was self-contained in regard to the presence in the experiment of standard suspensions set up along with the test suspensions. The convenience of the tinturometer as an instrument for measuring the content of bacterial suspensions is obvious, provided it has established its claim to sensitiveness. It is easily carried, easily cleaned, can be used with fluids coloured or uncoloured, and for suspensions over a very wide range of concentration. In the experiments which follow, the same procedure was followed throughout. Test tubes of the different fluid media in quantities of 10 c.c. were always sown with one drop of seed culture from the same dropping pipette. The seed culture was a 24-hour growth in Douglas tryptic mutton broth which I shall designate by the letters D. T. M. B. The control fluid media in all these experiments were a Witte peptone water (1 per cent Witte peptone and 0.5 per cent salt) to be called W. P. W. and the above mentioned Douglas broth. The sterilization of these media consisted in steaming at 95°C. for 30 minutes on 3 successive days. Growth was, unless otherwise stated, for 24 hours at 37°C. The culture was vigorously shaken up before proceeding to the estimation of growth. The figures given under the various experiments are tinturometer figures. The larger the figure, the less is the growth of organisms which has taken place—the less, in other words, is the light absorptive power of the suspension under test.

## EXPERIMENT NO. 1.

*To test the yield obtained by the use of various brands of peptone. Organism B. typhosus.*

Peptone.	1st trial.	2nd trial.	3rd trial.
W. P. W.	4.0	4.2	4.7
C.	3.1	3.0	3.2
F.	3.0	3.2	3.8
B.	2.8	3.2	3.6
M.	3.1	3.0	3.0
A.*	5.0	4.8	5.2
D. T. M. B.	0.5	0.5	0.6

\* This peptone did not, by any means, completely pass into solution, even on boiling. There remained flocculent solid matter which was filtered off.

## CONCLUSIONS.

(1) Douglas tryptic mutton broth far surpasses all peptone water media in the yield of bacteria given.

(2) The different brands of peptone used, when made up into peptone water, gave markedly different results as regards the 24-hour growth of *B. typhosus*. There is no reason to suppose, in accepting this conclusion, that *B. typhosus* is an exceptional type of organism.

(3) An indication is afforded that satisfactory tests for a bacteriological peptone would be (1) completeness of solubility in water on boiling; (2) a comparatively good growth of *B. typhosus* in peptone water medium after 24 hours. Other tests which have to be considered are freedom from fermentable sugar and utility for the indol test.

## EXPERIMENT No. 2.

*To determine the effect on growth of B. typhosus of concentration of Witte's peptone in a peptone water medium.*

Concentration of Witte's peptone.	READING OF GROWTH OBTAINED.		Reading of tinturometer for these fluids before sowing.
	I.	II.	
1% . . . . .	5.0	4.0	10
2% . . . . .	3.0	2.4	9.8
3% . . . . .	2.1	1.6	9.6
4% . . . . .	1.5	1.4	9.0
D. T. M. B. . . . .	0.7	0.5	—

## CONCLUSIONS.

(1) Increase in the concentration of Witte's peptone in peptone water affords, for 24 hours' incubation, a greater yield of organisms.

(2) The increased light absorptive effect of the peptone water with increased concentration of peptone has to be taken into account in giving expression to the growth values obtained, as this also shows increase. But with this taken into account the statement of increase of yield with increased concentration of peptone holds good.

## EXPERIMENT NO. 3.

To determine the effect of incorporation of certain sugars of varying concentration in peptone water upon the growth of *B. typhosus*. Incubation, 24 hours.

Medium.	Concentration of sugar.	TINTUROMETER READING.	
		I.	II.
W. P. W. . . . .	0	5.4	5.6
Glucose . . . . .	0.5%	1.9	1.4
Do. . . . .	1 %	2.2	1.8
Do. . . . .	2 %	1.8	1.5
Do. . . . .	4 %	2.0	1.9
Lactose . . . . .	0.5%	4.8	5.5
Do. . . . .	1 %	5.0	5.6
Do. . . . .	2 %	5.0	5.6
Do. . . . .	4 %	5.1	5.5
Mannite . . . . .	0.5%	1.6	1.5
Do. . . . .	1 %	1.8	1.9
Do. . . . .	2 %	1.7	1.7
Do. . . . .	4 %	2.3	1.5
D. T. M. B. . . . .	0	0.6	1.0
D. T. M. B. . . . .	0.5% glucose.	0.5	0.4

## CONCLUSIONS.

(1) Growth is less in simple peptone water than in peptone water containing sugar.

(2) Growth is only slightly, if any, better in non-fermentable sugar peptone water than in simple peptone water.

(3) Growth is markedly better in the case of a fermentable sugar like glucose than in non-fermentable sugars like lactose and mannite.

(4) There is no advantage as far as yield is concerned in increasing the concentration of fermentable sugar over 0.5 per cent.



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(5) A better growth is given by Douglas tryptic mutton broth without added sugar than in peptone water containing fermentable sugar.

(6) The growth in Douglas tryptic mutton broth containing 0.5 per cent of fermentable sugar is better than the growth in this medium without added sugar.

### EXPERIMENT NO. 4.

*To determine the effect of the incorporation of certain sugars of varying concentration in Witte's peptone water upon the growth of B. coli. Incubation, 24 hours.*

Medium.	Concentration of sugar.	Tinturometer reading.
W. P. W.	0	5.8
Glucose	0.5%	1.5
	1 %	1.4
	2 %	1.3
	4 %	1.4
Lactose	0.5%	1.9
	1 %	1.4
	2 %	1.6
	4 %	1.7
Mannite	0.5%	1.4
	1 %	1.4
	2 %	1.3
	4 %	1.6
D. T. M. B.	0	1.4

### CONCLUSIONS.

(1) Growth is less in simple peptone water than in peptone water containing sugar.

(2) Growth in the case of *B. coli* is markedly different from that of *B. typhosus* in lactose and mannite media. These sugars are fermentable by *B. coli* and not by *B. typhosus*. The inference is that markedly improved growth occurs in a sugar peptone water if the sugar is fermentable by the organism, over the growth in a non-fermentable sugar peptone water. If we admit that degree of growth of an organism in sugar peptone water is directly correlated with degree of power to ferment

the sugar used, we have in the tinturometer an instrument for measuring quantitatively sugar reactions.\*

(3) There is no evidence of advantage as far as yield is concerned of increasing the concentration of fermentable sugar over 0.5 per cent.

#### EXPERIMENT No. 5.

*To determine the effect of combining the minimal optimum amount (0.5 per cent) of fermentable sugar (lactose) with increasing concentrations of Witte's peptone on the yield of B. coli. Incubation, 24 hours.*

Concentration of Witte's peptone.								Concentration of sugar.	Tinturometer reading.
%								%	
1	.	.	.	.	.	.	.	0	5.8
2	.	.	.	.	.	.	.	0	3.5
3	.	.	.	.	.	.	.	0	2.6
4	.	.	.	.	.	.	.	0	1.8
1	.	.	.	.	.	.	.	0.5	1.3
2	.	.	.	.	.	.	.	0.5	0.8
3	.	.	.	.	.	.	.	0.5	0.6
4	.	.	.	.	.	.	.	0.5	0.5
D. T. M. B.	.	.	.	.	.	.	.	0	1.4

#### CONCLUSIONS.

(1) There is a distinct advantage as regards growth, in combining in peptone water medium the optimum amount of fermentable sugar (0.5 per cent) with the optimum amount of peptone over the growth obtained with the optimum amount of peptone alone.

(2) Growth of *B. coli* in lactose peptone water containing optimum amounts of lactose and peptone is greater than that in Douglas tryptic mutton broth.

\* The presence of acid as a result of fermentation of sugar can be shown by adding to the culture one drop of saturated aqueous neutral red solution. This solution can be kept in stock with the addition of chloroform as preservative.

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### EXPERIMENT NO. 6.

*To determine the effect of increase of quantity of seed on the yield of B. typhosus obtainable from Witte's peptone. The seed culture used was a 24-hours growth in Douglas tryptic mutton broth. Incubation, 24 hours.*

Amount of seed culture with which sown.						Tinturometer reading.
Drops.						
1	.	.	.	.	.	6.0
2	.	.	.	.	.	5.7
4	.	.	.	.	.	5.6
8	.	.	.	.	.	5.7

In this experiment it has to be remembered that the addition of sowing fluid dilutes to some extent the fluid sown, that the seed itself contributes something to the reading and that the broth of the seed culture may, in the larger quantities, contribute something to the nutritive value of the medium sown. In spite of these objections to the method of the experiment there is little evidence that in a peptone water fluid medium there is increase of growth with heavier sowing. There may be a certain amount of improvement with heavier sowing over very light sowing as indicated by the difference between the No. 1 tube and the rest.

### EXPERIMENT NO. 7.

*To show the effect of duration of incubation on the yield of B. typhosus and the effect of the presence of fermentable sugar on (1) the yield, (2) the viability of the organism with lengthened incubation.*

Medium I. = 1 per cent peptone; 0.5 per cent salt.

Medium II. = 1 per cent peptone; 0.5 per cent salt; 0.5 per cent glucose.

Medium III. = Douglas tryptic mutton broth.

Days of Incubation.*	MEDIUM I.		MEDIUM II.		MEDIUM III.	
	Tinturometer reading.	Viability.	Tinturometer reading.	Viability.	Tinturometer reading.	Viability.
1 . .	5.8	Alive	1.8	Alive	1.3	Alive
2 . .	4.8	Alive	1.8	Alive	1.3	Alive
3 . .	4.2	Alive	1.5	Alive	1.5	Alive
4 . .	4.0	Alive	1.6	Alive	1.4	Alive
5 . .	3.8	Alive	1.6	Dead	1.5	Alive
6 . .	3.8	Alive	1.6	Dead	1.5	Alive
7 . .	3.8	Alive	1.6	Dead	1.5	Alive
8 . .	3.7	Alive	1.5	Dead	1.5	Alive

\* Eight tubes of each medium were sown with the same quantity of seed culture. A certain amount of evaporation of medium with consequent concentration of growth has to be allowed for in drawing conclusions as to increase of yield.

## CONCLUSIONS.

(1) There is a considerable increase in the growth of 48 hours over 24 hours for *B. typhosus* in Witte peptone water and of 72 hours over 48 hours. The increase after that is not striking.

(2) There is increase with duration of incubation in glucose Witte peptone water but not of a striking character. The main growth has already taken place in 24 hours.

(3) There is little evidence in this experiment of increase of growth with duration of incubation in Douglas tryptic mutton broth. There is, if anything, evidence of the reverse, that is of lysis or digestion.

(4) The organisms are still alive in Witte peptone water and Douglas tryptic mutton broth after 8 days' incubation but are dead after 120 hours in the case of glucose Witte peptone water.

## EXPERIMENT NO. 8.

*To show the effect of age of medium on yield. A quantity of medium was made and tubed and kept for varying periods before use. The same capillary pipette was used for sowing all the tubes, one drop of seed culture (24 hours' growth in Douglas tryptic mutton broth) to 10 c.c. of medium.*

Age of medium hours.	Tinturometer reading.	Age of medium weeks.	Tinturometer reading.
24 . . . . .	0.7	2	0.8
48 . . . . .	0.8	3	0.9
72 . . . . .	0.8	4	1.0
96 . . . . .	0.7	5	0.8
120 . . . . .	0.8	6	0.8
144 . . . . .	0.8	7	0.8
168 . . . . .	0.8	8	0.9

## CONCLUSION.

There is little evidence of any effect on degree of growth due to age of medium in the case of nutrient broth. This conclusion, however, may not apply to solid media.

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## EXPERIMENT No. 9.

*To show the effect of addition of various substances to peptone water on the yield of B. typhosus. Incubation, 24 hours.*

### I.

#### CALCIUM CHLORIDE AND SODIUM CITRATE.

Substance or medium used.	Quantity. o/o	Tinturometer reading.
Calcium chloride . . . . .	0.025	5.8
	0.05	7.1
	0.1	7.0
Sodium citrate . . . . .	0.025	6.2
	0.05	7.4
	0.1	7.4
W. P. W. . . . .	—	5.8
D. T. M. B. . . . .	—	1.2

### II.

#### TRYPSIN.

*The trypsin used was the laboratory preparation—an extract of sheep pancreas with 30 per cent alcohol and containing hydrochloric acid as preservative.*

Substance or medium used.	Quantity. o/o	Tinturometer reading.
Trypsin . . . . .	0.2	4.2
	0.4	3.4
	0.6	3.1
	0.8	3.2
	1.0	3.1
	1.5	2.8
	2.0	2.9
W. P. W. . . . .	—	5.5
D. T. M. B. . . . .	—	0.9

The results show an improvement in growth of a distinct kind from the addition of trypsin to peptone water. No growth at all took place in pure trypsin, but a reading of 3.6 was obtained from a control medium

consisting simply of the addition of 1 c.c. trypsin to 9 c.c. normal salt solution.

## III.

## MEAT EXTRACTS.

Substance or medium used.	Quantity. %	Tinturometer reading.
Mutton extract in which 1 per cent peptone and 0.5 per cent salt were dissolved.	—	1.4
Lemco . . . . .	0.5 0.25	3.1 2.8
Essence of mutton . . . . .	0.5 0.25	4.8 4.5
W. P. W. . . . .	—	5.1
D. T. M. B. . . . .	—	1.0

## CONCLUSIONS.

(1) The addition of the salts, calcium chloride and sodium citrate, to peptone water does not, in the quantities used, produce an increase of yield of *B. typhosus* but rather the reverse.

(2) The addition of trypsin to peptone water results in an increased yield which is greater with greater concentration of the trypsin. The concentrated trypsin preparation did not itself give a growth, but it seems obvious that trypsin itself does form a medium for growth of organisms and may possibly stimulate growth.

(3) The addition of meat extract, whether in the form of mutton meat extract or of beef extract as in 'Lemco,' exerts a very definite effect on the growth of *B. typhosus* in peptone water. The greater effect was exerted by freshly prepared mutton extract. Essence of mutton produced a comparatively small effect—it may be due to age, presence of preservatives, or some other cause.

The experiments so far carried out have been done with Witte peptone water as a basis and control. A still better basis and control, if satisfactory, would be a simple 'Synthetic' medium, that is to say, a medium of known chemical composition. One of the simplest which has been used is composed of 0.4 per cent sodium ammonium phosphate and 1 per cent lactose in distilled water—the sodium ammonium phosphate being the only nitrogen source and the lactose the only carbon source for the growth and metabolism of bacteria.

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## EXPERIMENT No. 10.

*To show the effect of addition of various substances to a simple 'Synthetic' medium on the yield of various organisms. The letters S. S. M. are used to designate the simple 'Synthetic' medium. The reaction of this medium was faintly alkaline.*

Substance or medium used.	TINTUROMETER READING.		
	B. typhosus.	B. coli.	B. cholerae.
1. S. S. M . . . . .	8.4	5.0	9.2
2. S. S. M. + 1 per cent peptone . . . .	5.1	1.0	4.0
3. S. S. M. + 1 per cent peptone + 0.5 per cent sodium chloride.	5.2	0.9	3.8
4. 0.4 per cent sodium ammonium phosphate + 1 per cent lactose in mutton meat extract (faintly alkaline to litmus).	1.8	0.9	2.4
5. Same as 4 with the addition of 1 per cent peptone.	1.6	0.4	2.4
6. Same as 4 with the addition of 1 per cent peptone and 0.5 per cent sodium chloride.	1.5	0.3	1.8
7. Simple meat extract alone (faintly alkaline to litmus).	2.6	1.8	2.8
8. W. P. W. . . . .	5.4	4.9	4.3
9. D. T. M. B. . . . .	1.2	0.9	1.4

## CONCLUSIONS.

(1) The growth of *B. typhosus* and *B. cholerae* in simple 'Synthetic' medium is very slight. The growth of *B. coli*, as one would expect from its action on lactose, is much more marked.

(2) The growth of *B. cholerae* in simple peptone water is better than that of even *B. coli*. This is in accordance with the fact that peptone water is a selective medium for *B. cholerae*.

(3) The addition of peptone to simple 'Synthetic' medium increases the yield of all three organisms, most markedly of *B. coli*. Again, the effect of the presence of fermentable sugar has to be taken into account.

(4) The addition of sodium chloride to the peptone-synthetic medium only slightly, if at all, increases the yield.



(5) The growth of *B. coli* is as good in peptone-synthetic medium and in meat extract-synthetic medium as in Douglas tryptic mutton broth, but that of *B. typhosus* and *B. cholerae* is not so good.

(6) The growth of *B. coli* in peptone-meat extract-synthetic medium is even better than that in Douglas tryptic mutton broth—an effect once more doubtless of the presence of fermentable sugar. Probably the substitution of glucose for lactose in the ‘Synthetic’ medium would improve the growth of *B. typhosus* and *B. cholerae*, and the addition of either to Douglas tryptic mutton broth would improve the growth of *B. coli*.

(7) Meat extract alone affords a not unfavourable medium for the growth of bacteria.

(8) In the above experiment the main factors influencing growth are the presence of (a) fermentable sugar, (b) peptone, (c) meat extract.

THE DETERMINATION OF INCUBATION  
PERIODS FROM MARITIME STATISTICS,  
WITH PARTICULAR REFERENCE  
TO THE INCUBATION PERIOD  
OF INFLUENZA.

BY

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THE numerous reports which have been issued concerning the recent influenza pandemic, compiled with great care, and emanating from all parts of the world, provide a rich mine of information regarding the epidemiological features of the disease. In one of these ('Influenza and Maritime Quarantine in Australia,' Service pamphlet No. 18), Dr. F. H. Clumpston, Director of Quarantine of the Commonwealth of Australia, deals with the period of quarantine which should be enforced in order to prevent the introduction of the disease into a non-infected country. The report records many observations on the incidence, spread, and decline of epidemics on board ships. The figures

are of peculiar interest in that every member of these ship populations was under observation throughout the course of the epidemic.

The particular statistics with which we propose to deal in the present communication refer to the number of days after leaving port on which the first case of influenza appeared. We shall show how it is possible from statistics of this nature to deduce the mean incubation period of a disease, and also the expected frequency of occurrence of all incubation periods. That is to say, we shall calculate the relative numbers of cases which would be expected to have incubation periods of 1, 2, 3, . . . . days respectively.

The method is, of course, general, and can be applied to similar figures relating to any disease. We shall apply it, in the present instance, to the figures for influenza collected by Dr. Clumpston, but it must be clearly realised that too much stress should not be laid on numerical values obtained from a single set of observations.

The material with which we have to deal relates to 'all the interstate or coastal vessels which became infected between 1st January and 30th April, 1919.' These coastal vessels were 'constantly inspected from port to port, and were therefore under conditions 'such as to permit of more accurate observation' regarding the occurrence of even single cases than was possible in the case of overseas vessels. The ships varied in size from tug boats to liners of over 10,000 tons, and their crews and passengers ranged in number from 6 to 1,189 individuals. Of the total vessels examined, 92 were found to have been infected. 'There were 64 in which the incubation period appeared to be within one day after leaving port or during their stay in port, there were 17 in which the incubation period appeared to be two days, 5 in which it appeared to be three days, and 2 in which it appeared to be four days.' The first day is the day after the vessel leaves port, but it may also include a part of the day in which the vessel left port; thus in an extreme case, the so-called first day might refer to a period of 36 hours. We shall however in making numerical computations take the figures as they stand, that is to say, 64 on the first day, 17 on the second, 5 on the third, and 2 on the fourth day, but it must be fully realised that the accuracy of the numerical results which we shall obtain depends upon the validity of this simplification.

We shall also, in the first instance, assume that in each ship, only a single case of influenza was taken on board; and then later, by statistical grouping, we shall examine the validity of this assumption.

The problem presents certain points of interest:—

1. If the first case developed symptoms on the first day after leaving port, it need not necessarily have had a period of incubation of one day. The patient might have been infected 0, 1, 2, . . . . . days before leaving port. Similarly, if the first case developed symptoms on the second day out, the only incubation period which is excluded is that of one day—and so on.

2. That a case had an incubation period of, say, three days implies that he did not show symptoms on the first and the second days, or, in other words, the probability of an incubation period of three days is the probability that symptoms appeared *for the first time* on the third day.

3. We are thus thrown back to the consideration of a fundamental probability; which is the probability that a case showed symptoms on any particular day, irrespective of whether he did or did not show symptoms on the previous days.

4. In what follows the term 'case of influenza' is used to denote a person who ultimately developed symptoms. Persons, who, although infected, escaped the disease, are excluded.

Let  $p_r$  denote the fundamental probability to which we have referred. That is to say, let  $p_1, p_2, p_3, \dots, p_r$  denote the probabilities that a case showed symptoms on the 1st, 2nd, 3rd, . . . . .  $r$ th days respectively.

Then  $(1-p_1), (1-p_2), (1-p_3), \dots, (1-p_r)$  are the probabilities that he did not show symptoms on the respective days.

That a case showed symptoms *for the first time* on, say, the third day—or, in other words, that he had an incubation period of 3 days—implies that symptoms failed to appear on the first and second days, but appeared on the third. The probability of such an occurrence is then  $(1-p_1)(1-p_2)p_3$ .

Thus if  $Z_1, Z_2, Z_3, \dots, Z_r$  denote the probabilities of occurrence of incubation periods of 1, 2, 3, . . . . .  $r$  days respectively.

$$\left. \begin{aligned} Z_1 &= p_1, \\ Z_2 &= (1-p_1)p_2, \\ Z_3 &= (1-p_1)(1-p_2)p_3, \\ \text{and in general } Z_r &= (1-p_1)(1-p_2)\dots(1-p_{r-1})p_r. \end{aligned} \right\} (1)$$

If the first case after leaving port developed symptoms on the 1st day, he may have had an incubation period of 1 or 2 or 3 or . . . . .  $r$  days;

on the 2nd day, he may have had an incubation period of 2 or 3 or . . .  $r$  days;

on the 3rd day, he may have had an incubation period of 3 or 4 or . . .  $r$  days.

In other words, a first case with a

1 day incubation period can occur only on the 1st day,

2 days        "        "        "        "        1st or 2nd days,

3        "        "        "        "        1st or 2nd or 3rd days, etc.

Thus the total number of *ways* in which a first case can occur is

1 way, with an incubation period of 1 day,

2 ways,        "        "        "        "        2 days,

3        "        "        "        "        3 days, etc.

Now the probability of a first case occurring on any particular day is the sum of the probabilities of the ways in which it can occur on that particular day, divided by the sum of the probabilities of the ways in which it can occur on all the days.

Hence the probability of occurrence of a first case

$$\text{on the 1st day is } \frac{Z_1 + Z_2 + Z_3 + \dots}{1Z_1 + 2Z_2 + 3Z_3 + \dots},$$

$$\text{on the 2nd day is } \frac{Z_2 + Z_3 + \dots}{1Z_1 + 2Z_2 + 3Z_3 + \dots},$$

$$\text{and in general for the } r\text{th day it is } \frac{Z_r + Z_{r+1} + \dots}{\Sigma rZ_r}.$$

The frequencies of occurrence, where  $N$  is the total number of infected ships, and only a single case was taken on board, are—

$$F_1 = \frac{N}{\Sigma rZ_r} (Z_1 + Z_2 + Z_3 + Z_4 + \dots),$$

$$F_2 = \frac{N}{\Sigma rZ_r} (Z_2 + Z_3 + Z_4 + \dots),$$

$$F_3 = \frac{N}{\Sigma rZ_r} (Z_3 + Z_4 + \dots),$$

$$F_4 = \frac{N}{\Sigma rZ_r} (Z_4 + \dots);$$

and these from the Australian statistics are equal to 64, 17, 5, and 1 respectively.

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But the sum of probabilities of all possible incubations is certainty, i.e.,  $Z_1 + Z_2 + Z_3 + \dots = 1$ , hence we may write

$$F_1 = \frac{N}{\sum rZ_r} = 64.$$

$$F_2 = \frac{N}{\sum rZ_r} (1 - Z_1) = 17.$$

$$F_3 = \frac{N}{\sum rZ_r} (1 - Z_1 - Z_2) = 5.$$

$$F_4 = \frac{N}{\sum rZ_r} (1 - Z_1 - Z_2 - Z_3) = 2.$$

By simple algebra we have

$$Z_1 = 0.731,$$

$$Z_2 = 0.186,$$

$$Z_3 = 0.047,$$

$$Z_4 = 0.031.$$

Also introducing these values into equations (1) we find the fundamental probabilities

$$p_1 = 0.731.$$

$$p_2 = 0.699.$$

$$p_3 = 0.587.$$

$$p_4 = 0.937.$$

As the last two values are relatively unreliable on account of the small integral values from which they are calculated, it seems reasonable to assume, as a first approximation, that  $p_r$  is constant within the range of observation.

Equation (1) can now be reduced, and we have  $Z_r = p(1-p)^{r-1}$ . Also as  $\sum rZ_r = p\{1 + 2(1-p) + 3(1-p)^2 + \dots\}$

$$= p \left\{ \frac{1}{p} + \frac{1-p}{p^2} \right\} = \frac{1}{p},$$

$$\begin{aligned} \text{and } (Z_r + Z_{r-1} + \dots) &= p(1-p)^{r-1} + p(1-p)^r + p(1-p)^{r+1} + \dots \\ &= p(1-p)^{r-1} \left\{ 1 + (1-p) + (1-p)^2 + \dots \right\} \\ &= p(1-p)^{r-1} \frac{1}{p} = (1-p)^{r-1} \end{aligned}$$

the general form of equations (2) becomes—

$$F_r = Np(1-p)^{r-1} = NZ_r \dots \dots \dots (3)$$

The best value of  $p$  can now be obtained by moments,

$$\text{for } \Sigma rF_r = N \Sigma rZ_r = \frac{N}{p}$$

$$\therefore p = \frac{N}{\Sigma rF_r}$$

The calculation is as follows:—

	$F_r$	$rF_r$
$r = 1$	64	64
$= 2$	17	34
$= 3$	5	15
$= 4$	2	8
$N =$	88	$\Sigma rF_r = 121$

$$\text{hence } p = \frac{88}{121} = 0.727.$$

Consequently by (3)—

	Observed.	Calculated to nearest integer.
$F_1 = 63.98$	64	64
$F_2 = 17.47$	17	17
$F_3 = 4.77$	5	5
$F_4 = 1.30$	2	2
$F_5 = 0.36$	0	

The values of  $Z_r$  are

$$Z_1 = 0.727,$$

$$Z_2 = 0.189,$$

$$Z_3 = 0.054,$$

$$Z_4 = 0.014.$$

The mean incubation period from these figures is 32.71 hours with a probable error of about one hour.

The validity of the assumption that only one case was introduced into each ship may now be tested. If more than one case were introduced the resulting epidemic would probably be larger than if only one were taken on board. Consequently if the statistics were divided into classes according to the total number of cases which occurred, the non-validity of our assumption would be expected to declare itself in perturbations amongst the higher classes. The following figures show that there is little evidence of this, and that consequently the assumption may be accepted. This is the only way in which



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differences in size, amongst the populations of the ships can affect the argument.

	Calculated.	Observed.	Calc. to nearest integer.	
Ships with one case only	F <sub>1</sub>	20.17	21	20
	F <sub>2</sub>	6.30	5	6
	F <sub>3</sub>	1.94	3	2
	F <sub>4</sub>	0.60	1	1
<hr/>				
" " 2-5 cases	F <sub>1</sub>	22.11	21	22
	F <sub>2</sub>	3.99	5	4
	F <sub>3</sub>	0.72	1	1
	F <sub>4</sub>	0.13	0	0
<hr/>				
" " 6-10 cases	F <sub>1</sub>	8.65	9	9
	F <sub>2</sub>	1.85	1	2
	F <sub>3</sub>	0.40	1	0
<hr/>				
" more than 10 cases	F <sub>1</sub>	8.0	8	8
	F <sub>2</sub>	2.67	3	3
	F <sub>3</sub>	0.8	0	1
	F <sub>4</sub>	0.3	1	0

In the above it has been assumed that variations in the chance of an individual acquiring infection were negligible during the four days prior to leaving port.

#### SUMMARY.

(1) From the times of occurrence of first cases of influenza, after the departure from port of the ships under consideration, it appears that the probability of a patient showing symptoms (irrespective of whether he showed them previously or not) does not significantly vary during the first four days of his infection.

(2) If this probability be taken as constant during these days, then the following results are obtained:—

(a) Ships in which the first case appeared

	Observed.	Calculated to nearest integer.
On the 1st day after leaving port	61	64
" " 2nd " " " "	17	17
" " 3rd " " " "	5	5
more than 3 days " "	2	2

(b) It also follows that of 100 cases of influenza, it is to be expected that 72·7 would have incubation periods of 1 day

18·9	“	“	“	“	“	2 days
------	---	---	---	---	---	--------

5·4	“	“	“	“	“	3 “
-----	---	---	---	---	---	-----

1·4	“	“	“	“	“	4 “
-----	---	---	---	---	---	-----

the mean incubation period being 32·7 hours.

(3) If the probability of a case being infective be related to the probability of symptoms being apparent, then the initial period of non-infectivity in influenza must be of short duration, which is a point of considerable epidemiological significance.

# EVIDENCE REGARDING THE IMMUNITY CONFERRED BY AN ATTACK OF INFLUENZA WITH A STUDY OF THREE LOCAL EPIDEMICS.

BY

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WITH MATHEMATICAL NOTE

BY

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INFLUENZA appeared in Calcutta as an epidemic in July 1918, and again in November of the same year: and during the first quarter of 1919, many patients were still being admitted to the city hospitals for this disease.

In May 1919, I was able to collect statistics relating to the July epidemic in three different communities in Calcutta and its neighbourhood, viz., the Gourepore Jute Mills; the Central Jail, Alipore; and the Presidency Jail, Calcutta.

An analysis of these figures adduces strong evidence that immunity is conferred by one attack of influenza.

TABLE I.

*Influenza at the Gourepore Jute Mills.*

Cases of influenza.				Hindus.	Mohamedans.
July 9th to 31st, 1918	..	..	..	432	295
August 1st to December 31st, 1918	..	..	..	22	9
January 1st to 31st, 1919	..	..	..	20	4
February 1st to 28th, 1919	..	..	..	0	35
March 1st to April 30th, 1919	..	..	..	0	0
				474	343
TOTAL POPULATION	..	..	..	..	about 3,500.
MORTALITY	..	..	..	..	less than 1%.

No one had a second attack during this period.

In the mild recrudescence of February 1919, 26 of the 35 cases (all Mohamedans) occupied rooms with persons who had had one attack of influenza; none of the latter contracted the disease a second time.

TABLE II.

*Influenza at the Central Jail, Alipore.*

Date.	Admissions into Hospital.	Deaths.	Daily average number of prisoners in Jail.	Incidence. %	Mortality. %
July 1918 ..	227	3	1563	14.5	1.1
August 1918 ..	0	0	....	..	..
September 1918 ..	0	0	....	..	..
October 1918 ..	18	0	1547	..	..
November 1918 ..	10	1	1502	..	..
December 1918 ..	7	1	1486	..	..
January 1919 ..	8	1	1453	..	..
February 1919 ..	3	1	1433	..	..
March 1919 ..	4	..	1471	..	..
April 1919 ..	0	..	....	..	..

Of the 227 influenza cases in July 1918, 172 were still in the jail at end of April 1919. Only one of these had a second attack and this occurred in November 1918.

TABLE III.

*Influenza at the Presidency Jail, Calcutta.*

Date.	Admissions into Hospital.	Deaths.	Daily average number of prisoners in Jail.	Incidence. %	Mortality. %
July 1918 ..	245	1	1806	13.5	0.4
August 1918 ..	1	1	1787	..	..
January 1919 ..	3	3	1827	..	..

In this jail there were no cases of influenza from August to December 1918, nor from February to the end of April 1919. The three cases in January 1919 were transferred from Howrah Jail while suffering from influenza.

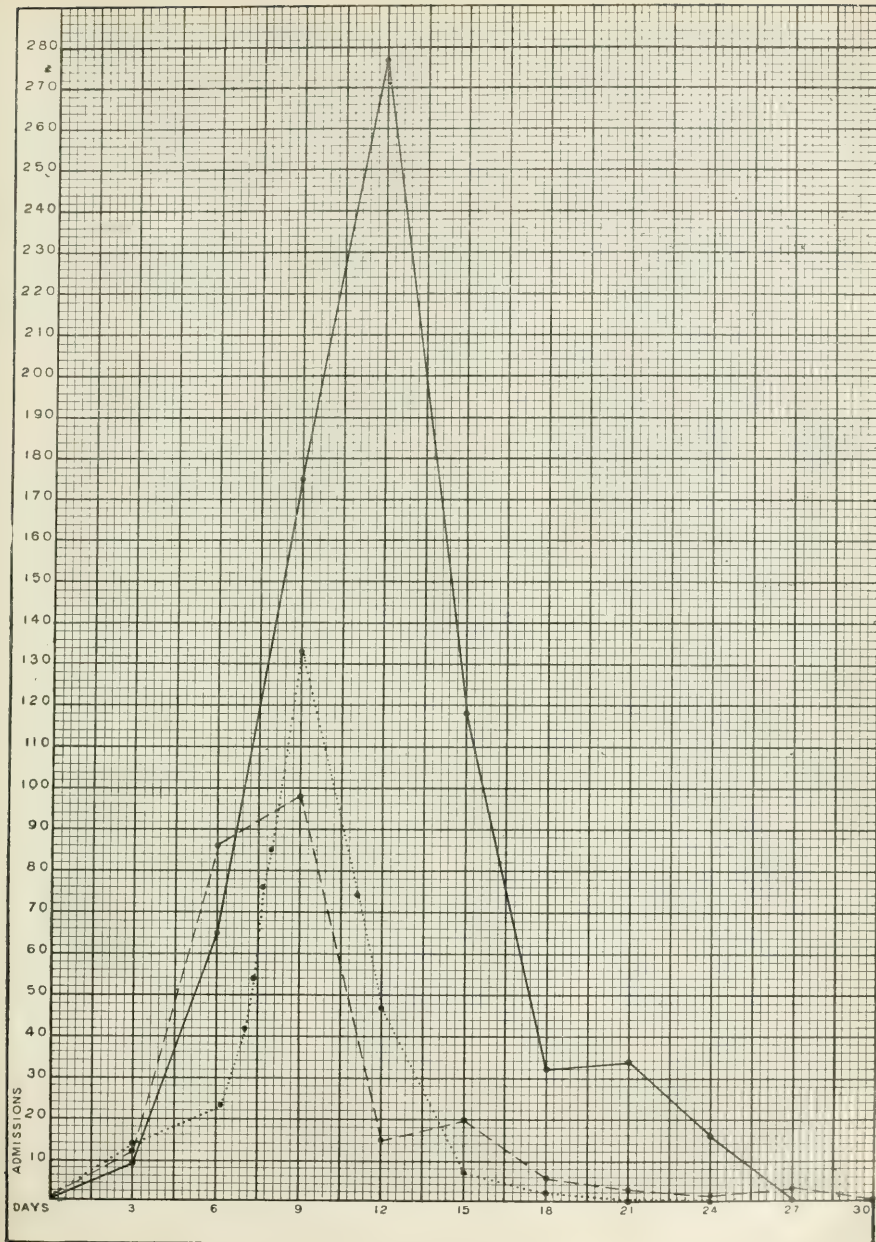
Of the 245 cases of influenza in July, four were readmitted to hospital in the same month, with a second attack, or with what may be more fairly termed a relapse.

Case i, admitted	July 9th,	discharged	July 12th.
readmitted	„ 18th,	„	August 5th.
Case ii, admitted	„ 9th,	„	July 12th.
readmitted	„ 22nd.	„	„ 25th.
Case iii, admitted	„ 9th,	„	„ 12th.
readmitted	„ 31st.	„	August 19th.
Case iv, admitted	„ 12th,	„	July 18th.
readmitted	„ 29th,	„	August 7th.

TABLE IV.

*Daily admissions to hospital during the influenza epidemic of July 1918.*

Day.	Gourepore Jute Mills.	Central Jail, Alipore.	Presidency Jail, Calcutta.
1	6 (July 9th)	7 (July 9th)	4 (July 5th)
2	1	3	9
3	3	4	0
4	11	13	30
5	17	10	31
6	37	0	25
7	40	23	17
8	31	28	47
9	104	82	34
10	110	29	0
11	104	13	10
12	63	5	5
13	52	0	11
14	40	6	5
15	26	1	4
16	16	2	3
17	6	0	0
18	10	0	3
19	6	0	2
20	22	0	1
21	6	0	0
22	8	1	1
23	8	0	0
24	0	0	0
25	0	0	2
26	0	0	0
27	0	0	1
	727	227	245
Average daily population	3500	1583	1895



----- PRESIDENCY JAIL, CALCUTTA,

———— GOUREROO JUTE MILLS

## SUMMARY.

In the Gourepore Jute Mills the population may be considered, for all practical purposes, as a fixed one. The employés live there with their wives and families; they are well housed and not overcrowded; there is a pure water supply and a good dispensary. The population of February 1919 was almost entirely composed of the same individuals who were there during the epidemic of July 1918. Influenza occurred in the district in December 1918, but there were very few cases in the mills. During the first week in February 1919, 35 Mohamedans were attacked, but no Hindus, and this mild epidemic was over by February 10th.

None of the employés who suffered from influenza in July had a second attack, in spite of the fact that many of them were direct or indirect contacts of infected persons, during the recrudescence in February, and some actually lived in the same rooms with influenza patients during their illness.

In the Alipore Central Jail and the Presidency Jail, Calcutta, the community consists of prisoners who are serving long sentences, and the population is not a rapidly changing one. In the former jail 172 out of the 227 prisoners who had influenza in July 1918, were still in jail at the end of April 1919. Only one of these had a second attack, November 1918, although from October 1918 to March 1919 there were a certain number of influenza cases in the jail.

The evidence here strongly suggests that immunity is acquired by one attack of influenza, and that it lasts for at least nine months.

The other point brought out by a study of these epidemics is the remarkably rapid progress of the disease and its almost as rapid decline. The figures in Table IV and the chart showing the total admissions every three days, reveal the close similarity of the admission curves in the three different institutions.

## MATHEMATICAL NOTE.

The problem contained in the statistics of the three communities detailed in Tables I, II, and III of Captain Malone's paper is an example of a type, with which I have already dealt in various papers published in this journal (Vol. 2, page 882; and Vol. 3, pages 266, 271, and 667).

It is in this instance the determination of the degree in which the probability of an attack occurring is reduced by the effects of previous attacks.



If  $v_x$  denote the number of persons who have suffered from  $x$  attacks, then for variations in  $v_x$  during the time  $dt$  we have :

$$\frac{dv_x}{dt} = \mathfrak{S}_{x-1} v_{x-1} - \mathfrak{S}_x v_x,$$

where  $\mathfrak{S}_x$  is the probability that an individual, who has suffered from  $x$  attacks, may experience a further attack.

If we assume, as an approximation, that  $\mathfrak{S}_x = b - cx$ , the solution of

the equation is  $v_x = N \frac{b}{c} \left( \frac{b}{c} - 1 \right) \dots \left( \frac{b}{c} - x + 1 \right) \frac{\left( \frac{\mu}{\nu} - 1 \right)^x}{x!} \left( \frac{\nu}{\mu} \right)^{b/c}$ .

Where  $N$  is the total population,  $\mu$  is the mean number of attacks per individual and  $\nu$  is the square of the standard deviation. Also  $c_b = \frac{\nu - \mu}{\mu^2}$ .

It is to be noted that in obtaining the above solution time factors which act equally on the whole population have been eliminated. Thus, for example, variations in the degree of risk during the period under review do not affect the solution.

1. At the Gourepore Jute Mills, between July 9th, 1918, and February 28th, 1919, there were in all 817 admissions, amongst 3,500 persons, no one of whom was admitted on two occasions. We have then  $N=3500$ ,  $v_1=817$ . Hence  $\mu=0.2334$ ,  $\nu=0.1789$ , and  $\frac{c}{b}=1$ . From these, by our equation, we find :

				Observed.	
Individuals admitted 0 times	2682.7	..	..	2863	
„ „ 1 time	817.3	..	..	817	
„ „ 2 times	0	..	..	0	

2. At the Central Jail, Alipore, 227 cases of influenza were admitted in July 1918, out of a total population of 1563 individuals. Of the 227 cases admitted 172 remained under observation until April 1919, only one of these had a second attack and this occurred in November 1918. We may legitimately assume that if the 227 corresponded to a total of 1563, then the 172 of whom we have full record, corresponded to a total of 1184.

We have then  $N=1184$ ,  $v_1=170$ ,  $v_2=1$ . Hence  $\mu=0.14527$ ,  $\nu=0.12586$ , and  $\frac{c}{b}=0.92$ . Hence by our equation we find :

				Observed.	
Individuals admitted 0 times	1013.1	..	..	1013	
„ „ 1 time	169.8	..	..	170	
„ „ 2 times	1.1	..	..	1	

3. In dealing with the figures collected from the Presidency Jail, Calcutta, we shall refer only to the figures for July 1918, as after this period the population cannot be considered to have been at risk.

We have  $N=1805$ ,  $v_1=237$ ,  $v_2=4$ . Hence  $\mu=0.135734$ ,  $\nu=0.121742$ , and  $\frac{c}{b}=0.759444$ . From these we find:

				Observed.
Individuals admitted	0 times	1564.1	..	.. 1564
„	„ 1 time	236.7	..	.. 237
„	„ 2 times	4.3	..	.. 4

It would thus appear that amongst the three communities the values of  $\frac{c}{b}$  are consistently high. We may interpret them relatively as follows. If the chance of getting a first case be taken as unity then the chance of an individual experiencing a second attack was

At Gourepore  $1-1.00=0$

At Alipore  $1-0.92=0.08$

At Calcutta  $1-0.76=0.24$

The three groups of statistics are consistent with the view that a high degree of immunity supervened on the first attack. It is however to be remarked that the above analysis does not take into account one fact that a sick person is segregated during the period of his illness and that, consequently, during that period, he is not subjected to the same risk as the rest of the community. Had the epidemics of the autumn of 1918 and the spring of 1919 been more intense, the value of the results of their statistical analysis by the above method would have been enhanced.

# PASTEURELLOSIS IN RABBITS, FOLLOWING THE INTRAVENOUS INJECTION OF INFLUENZA BACILLI.

BY

CAPTAIN R. H. MALONE, M.D., I.M.S.

[Received for publication, June 18, 1919.]

ON May 30th six rabbits were inoculated intravenously with live cultures of *B. influenzae* with the object of preparing high titre agglutinating sera. The strains used were 142, 206, 241, 262, 263, and M 37.

Next day they seemed fairly well but were rather drowsy.

On June 2nd Rabbit 142 became very ill. He was very weak, in attempting to walk often fell, and his head kept constantly wagging forwards and backwards. This was unexpected as he had already received three doses of live cultures of influenza bacillus given intravenously on May 4th, 11th, and 18th, without showing any ill effects. His blood serum, moreover, on May 25th possessed well-marked agglutinative properties.

He was chloroformed when in a dying condition, and a post-mortem examination made. The organs were markedly congested and there was excessive fluid in the pleural, pericardial, and peritoneal cavities, and at the base of the brain; but no pus. The brain, blood, liver, spleen, and kidneys yielded pure or almost pure cultures of a non-motile, Gram-negative cocco-bacillus, some resembling plump bacilli, others elongated cocci often in pairs. The lungs also contained this organism in abundance.

The cultures from the brain and blood were plated out on 2.5 per cent pigeon's blood agar. On this medium the colonies were round, transparent and convex with a distinct elevation in the centre, and resembled those of *Bacillus influenzae* so closely that I considered that

I was dealing with that organism, in spite of the fact the cocco-bacilli seemed rather plumper and shorter than freshly isolated influenza bacilli.

Rabbits 206, 250, and M 37 died on June 1st, and 263 died on June 2nd.

The pathological and bacteriological findings were identical with those of Rabbit 142, although none of the latter had developed any signs of brain affection.

Pure cultures of a Gram-positive cocco-bacillus were obtained from the blood of all these animals and planted on 2·5 per cent pigeon's blood agar, 2·5 per cent sheep's blood agar, plain agar, plain broth, glucose and lactose broth, and litmus milk.

On plain broth uniform turbidity of the medium was produced; no acid or gas was produced in glucose or lactose broth; milk was neither clotted, peptonized nor altered in reaction. The growth was as luxuriant on plain agar as on agar containing blood. Experiments showed this organism to be highly pathogenic for pigeons, and mice, as well as rabbits. Further it was not agglutinated by any of five anti-influenza sera (including the agglutinating serum produced by strain 142).

These characteristics conclusively proved that the organism was not Pfeiffer's bacillus. The cultures were recognised by Dr. G. C. Chatterji as belonging to the *Pasteurella* group of organisms and in a paper published by him (*Indian Medical Gazette*, Volume XLV, 1910, page 45), further details with regard to the cultural and pathogenic characters of one of the members of this group will be found.

On June 3rd, Rabbit 241 developed conjunctivitis in the right eye with a thick creamy white exudate. The pus contained a large number of the *Pasteurella* organism together with some *Staphylococcus albus* and a Gram-positive diphtheroid bacillus.

On June 6th, a rabbit which had not been inoculated died. Autopsy revealed marked congestion of the organs, dilation of the right heart, purulent pleurisy and pericarditis, 'nut-meg' liver and excessive fluid in the peritoneal cavity and at the base of the brain. Blood cultures showed *Pasteurella* and *Staphylococcus aureus*. The purulent exudates contained a mixture of organisms, *Pasteurella* and Gram-positive cocci predominating.

On June 10th, cultures were made from the nasal secretions of seven rabbits which had been in contact with those which had died of *Pasteurella* infection. In five cases the *Pasteurella* organism was

recovered (one in almost pure culture). None of these animals showed any signs of illness.

These observations are referred to as possibly having some bearing on the question of influenza and post-influenzal infections in man.

Fourteen rabbits apparently in good health were living under the same conditions in an open space in the Laboratory, about 12 ft.  $\times$  6 ft. Six were inoculated with living cultures of influenza bacilli, eight were not inoculated. All of the former developed Pasteurella infection (four died, one was chloroformed in a dying condition, and one had conjunctivitis). Of the latter, one died of a mixed infection in which Pasteurella played a part.

These, more or less, accidental findings suggest that the influenza bacillus (usually considered to be non-pathogenic for lower animals) may so lower the resistance of rabbits as to render them susceptible to infection by other organisms. They parallel the observations that in man the mortality from diseases such as tuberculosis and malaria have increased as a result of the influenza epidemic and, if the Pasteurella group be normal inhabitants of the rabbit, suggest a possible explanation for the occurrence of Group IV pneumococcus in such a large proportion of the cases of influenzal pneumonia.

Incidentally two other points were brought out: (1) The value of agglutination as a confirmatory test when one is dealing with organisms resembling the influenza bacillus. (2) The danger of overlooking intercurrent infections in inoculated animals, and the cautious attitude one should adopt in attributing loss of weight, rise of temperature, and other signs of illness to the direct action of the inoculum.

# REPORT ON AN EPIZOOTIC DISEASE AMONG CALVES AT THE AMARA DAIRY FARM.

BY

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AND

CAPTAIN G. SHANKS, I.M.S.

[Received for publication, June 6, 1919.]

IN December 1918, attention was directed to the high mortality amongst young calves at the Amara Dairy Farm and Captain C. J. R. Lawrence, R.A.V.C., was sent from Baghdad to investigate the cause.

Captain Lawrence made post-mortem examinations of 10 calves, in all of which he found varying degrees of enteritis. He took material for culture from 8 of the 10 calves, and from 5 of these he obtained an organism of the paratyphoid Gaertner group. The investigation, which is the subject of this report, was carried out at the Central Laboratory, Amara, under instructions from Director of Medical Services, Mesopotamian Expeditionary Force, and its main object was to determine the relationship of the calf bacillus to the bacilli of the paratyphoid Gaertner group which are the cause of enteric fever in man.

The Central Laboratory undertook the post-mortem examinations of the calves and the bacteriological and serological work. As regards the clinical aspects of the disease, the officer in veterinary charge of the dairy farm, Captain Scott, R.A.V.C., informed us that there were no very definite symptoms associated with the infection and in the majority of cases the illness was not recognised until a few hours before the death of the animal.

The disease, at least in its severe fatal form, appears to affect only young calves, from one to three weeks old. The investigation may be described under the following headings:—

1. Post-mortem examinations including the taking of cultures.
2. Study of the morphological and cultural characters of the organisms isolated.

3. Determination of the identity of bacilli isolated from different calves.
4. A comparison of the calf organism with the available organism of the *Salmonella* group by means of agglutination and absorption tests.
5. Agglutination of the calf bacillus by the sera of calves and dams.
6. Cultural examination of the blood and milk of dams of healthy and infected calves.
7. The pathogenicity of the organism for calves and laboratory animals.
8. An attempt to determine whether the calf disease is due to a filtrable virus.
9. Summary and conclusions.

#### 1. POST-MORTEM EXAMINATIONS.

Post-mortem examinations were made on 22 calves: 14 showed intestinal lesions; in 8 no gross lesions were found. Bacilli of the paratyphoid Gaertner (*Salmonella*) group were isolated from 16 calves, *viz.*, from all the calves with intestinal lesions and from 2 calves without such lesions. A detailed account of the post-mortem findings in two young calves is given below:

##### *Calf No. 3.*

Received dead from the Amara Dairy Farm on 23-1-19.

*Post mortem.*—Lungs and heart .. Normal.

Liver	..	„
Spleen	..	„
Stomach	..	„

*Intestine.*—The small intestine down to a point 2 feet above the cæcum shows no gross lesions. At this point small shallow pin-head ulcers in the mucosa appear. As the cæcum is approached, the ulcers become more numerous and larger. From 8 inches above the ileo-cæcal valve to and including the valve the entire mucous membrane is necrotic and covered with a yellow sloughy material.

The mucous membrane shows a few shallow erosions.

*Cæcum.*—The mucous membrane of the first 2 inches shows isolated shallow ulcers. Four inches below the valve the mucous membrane for



a length of 4 inches is necrosed and covered with a slough. The wall of the bowel in this situation is thickened and the peritoneal surface is deeply congested. Immediately below this area of necrosed mucous membrane, only discrete shallow ulcers are found. These extend to about  $2\frac{1}{2}$  feet below the valve. The bowel below this shows no ulceration.

The coils of intestine in the neighbourhood of the cæcum are matted together with recent lymph. There is no free fluid in the abdomen.

*Mesenteric lymph glands*.—Are large, soft, and much congested, some of them contain a thin purulent material. A bacillus of the paratyphoid Gaertner group was isolated from the blood, liver, spleen, mesenteric glands, and scrapings from the intestinal ulcers.

*Calf No. 10. (Age 10-15 days.)*

Received from the Amara Dairy Farm in a dying condition on 27-1-19. Died in the laboratory. Post-mortem immediately after death.

Lungs and heart .. Normal.

Liver .. "

Spleen .. Congested and soft.

Acute general peritonitis with much fluid in the peritoneal cavity.

A portion of small intestine, 3 feet in length, about 6 feet above the ileo-cæcal valve is extremely congested and in places thickened. It shows one perforation and many thin spots. The mucous membrane of this part of the gut is almost completely ulcerated with general thickening, but, in other places, thinning of the gut wall. Below this area the ileum is congested and the mucous membrane shows many small and hollow ulcers. The cæcum and upper 2 feet of the colon also show numerous shallow ulcers in the mucous membrane.

*Mesenteric glands*.—Enlarged, congested, and necrotic. Some of them contain pus.

*Cultures*.—An organism of the paratyphoid Gaertner group was isolated from the heart blood, bile, liver, spleen, and mesenteric lymph glands. The post-mortem findings in the other infected calves conformed generally to those above described. The intestinal lesions exhibited a rather striking localisation in the lower ileum, cæcum, and upper part of the colon. When, as sometimes happened, a more extensive tract of the bowel was involved, the more severe lesions were found in the situations just mentioned.

One calf with extensive lesions of the intestine had also several large ulcers in the stomach. A paratyphoid-like bacillus was isolated from 2 calves which showed no intestinal lesions. One of these, calf No. 9, from whose blood the bacillus was recovered, showed no gross lesions except a few grey nodules in some of the mesenteric lymph glands and marked pallor of all the tissues. The other calf, No. 22, showed no lesions except enlarged and congested mesenteric lymph glands. The bacillus was recovered from the bile and mesenteric lymph glands.

## 2. MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE BACILLUS ISOLATED FROM CALVES.

The bacillus is indistinguishable as regards its microscopic appearances and staining reactions from the paratyphoid B or typhoid or paratyphoid A bacillus.

*Cultural characters.*—In broth it grows abundantly, frequently producing a pellicle on the surface.

On agar it forms a dense white growth which is not characteristic.

*Action on carbohydrates.*—It forms acid and gas in glucose, mannite, and dulcitate media, but does not produce either acid or gas in lactose or saccharose.

*Litmus milk.*—In litmus milk it produces no change, or slight acidity at first. After 6–10 days the milk becomes strongly alkaline.

It will be seen that the cultural characters of the calf bacillus, in so far as they have been studied, conform to those of the paratyphoid B bacillus and the other members of the *Salmonella* group (*i.e.*, the group of organisms which have the morphological and cultural attributes of the bacillus of hog-cholera). Bainbridge, making use of Castellani's absorption reaction, came to the conclusion that the *Salmonella* group comprised only 3 distinct organisms, *viz.* :—

(I) *Bacillus paratyphosus* B.

(II) <i>Bacillus ærtrycke</i>	...	<i>Synonyms</i> : <i>B. of hog-cholera.</i> <i>B. suipestifer.</i> <i>B. cholerae suis.</i>
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(III) *Bacillus enteritidis* Gaertner.

In addition to these 3 organisms, the paratyphoid bacillus isolated from human cases of enteric fever, liver abscess, etc., in Mesopotamia,

provisionally named 'Inagglutinable paratyphoid B' or 'paratyphoid C,' must now be included.

### 3. IDENTITY OF BACILLI FROM DIFFERENT CALVES.

The investigation was next directed to determine by means of agglutination and absorption tests whether the bacilli isolated from different calves were identical species.

Agglutinating sera were prepared for several of the calf bacilli by highly immunising rabbits with small doses of live broth cultures, according to the method recommended by Bainbridge (quoted in Bessen's 'Practical Bacteriology,' p. 436). By this method sera with high specific titres (1-12,000 to 1-125,000) for the calf bacilli were obtained.

The agglutinations were carried out in small (Dreyer's) tubes using emulsions in formalinised saline of 20 hours' agar cultures of the bacilli. The tubes were kept in a water bath at 55°C. for 2 hours and readings were taken after standing for half an hour after removal from the bath.

*Absorption tests.*—The technique employed was essentially that adopted by Bainbridge in his work in the differentiation of the paratyphoid bacilli which is summarised in Bessen's 'Practical Bacteriology,' p. 436.

Table I shows the results of cross agglutination tests with sera, prepared by immunising rabbits with bacilli isolated from calves 3, 5, 6, and 4 (Lawrence).

It will be seen that each calf bacillus immune serum had almost the same titre for all the calf bacilli against which it was tested.

TABLE I.  
*Cross agglutination tests with calf bacilli sera.*

Serum.	AGGLUTINATION LIMITS FOR					
	C. 3.	C. 4.	C. 5.	C. 6.	C. 21.	C. 4 Lawrence.
Bacillus C. 3	125,000	83,000	..	..	..	125,000
Bacillus C. 5	..	..	50,000	50,000	..	50,000
Bacillus C. 6	..	12,500	..	8,300	..	12,500
Bacillus C. 4 (Lawrence)	50,000	50,000	..	..	..	50,000

Table II shows the effect of absorption of 2 calf sera by their own bacilli. The agglutinins for their own as well as for other calf bacilli are almost completely removed.

TABLE II.  
*Absorption tests with calf bacilli sera.*

Serum.	AGGLUTINATION LIMITS FOR				
	C. 3.	C. 4.	C. 5.	C. 6.	C. 4 Lawrence.
Bacillus C. 3 (original titre)	125,000	83,000	..	..	125,000
Absorbed with bacillus	500	500	..	..	500
Bacillus C. 5 (original)	..	..	50,000	50,000	50,000
Absorbed with bacillus C. 5	..	..	500	500	500

From the above tests it may be concluded that the bacilli isolated from different calves are identical species.

#### 4. COMPARISON OF THE CALF ORGANISM WITH THE ORGANISM OF THE SALMONELLA GROUP.

Having proved the identity of the bacilli isolated from different calves, the next stage in the inquiry that suggested itself was to make a comparative study, by means of agglutination and absorption tests, of the calf organism and known paratyphoid B ærtrycke and Gaertner strains.

Unfortunately, however, neither a proved ærtrycke nor a Gaertner bacillus was available. We had some Gaertner immune serum, about 2 years old, but not having a Gaertner bacillus to test it against, the results obtained with it are not reliable. It was therefore decided to try whether the calf bacillus could be identified with any of the organisms of the paratyphoid Gaertner group of which we had cultures.

Those were:—

1. A paratyphoid bacillus originally received from Lieut.-Colonel Ledingham, R.A.M.C., which is agglutinated up to titre by a specific paratyphoid B serum.

2. Four strains of a paratyphoid B-like bacillus which were isolated from the blood of human cases of an enteric-like fever. Two of these

strains, Nos. 782 and 3414, were isolated at Baghdad, and two, Nos. 1517 and 1536, in Amara. This bacillus was originally called by us an inagglutinable paratyphoid B, as it failed to agglutinate with B serum, at all events when first isolated. Subsequent work carried out at the Central Laboratory, Baghdad, proved these strains to be identical and to differ from the paratyphoid B bacillus. This has been confirmed here. The bacillus has been provisionally named *B. paratyphosus* C.

3. Three strains of a paratyphoid-like bacillus isolated from guinea pigs during an epizootic in this laboratory.

*Comparison of the calf bacillus with the paratyphoid B bacillus.*

Table III shows the agglutination of the calf bacilli and of the paratyphoid B bacilli by a paratyphoid B immune serum and by sera prepared from the calf bacilli. The titre of the paratyphoid B serum varies for different calf bacilli from less than 1-200 to over 1-3200 (limit not determined). On the other hand, the calf bacillus sera, which agglutinate the homologous bacilli in high dilutions (50,000 and 125,000), have relatively very low titres (250-1600) for the paratyphoid B bacillus.

TABLE III.  
*Cross agglutination test.*

Serum.	AGGLUTINATION LIMITS FOR									
	Para. B.	C. 3.	C. 4.	C. 5.	C. 6.	C. 8.	C. 9.	C. 10.	C. 22.	
Paratyphoid B. (1:2PM.)	3,200	3,200	200	3,200	200	200	200	200	3,200	
Calf 3 . . .	1,600	125,000	83,000	..	..	..	..	..	..	
Calf 4 . . .	250	..	..	..	..	..	..	..	..	
Calf 5 . . .	10,000	..	50,000	50,000	..	..	..	..	..	

The effect of absorbing paratyphoid B serum by the homologous bacillus and by calf bacilli is shown in table IV. The paratyphoid B bacillus removes most of the agglutinin both for B and the calf strain.

On the other hand, absorption of the same serum with calf bacilli does not affect the B agglutinins, but almost completely removes those for the calf bacilli.

TABLE IV.  
*Absorption tests.*

Serum.	AGGLUTINATION LIMITS FOR	
	B. para. B.	C. 5.
B para. B (original titre) . . . . .	> 3,200	> 3,200
Absorbed with B. para. B . . . . .	500	< 250
Absorbed with bacillus C. 4 . . . . .	> 3,200	..
Absorbed with bacillus C. 5 . . . . .	> 3,200	250

The marked difference of titre of the calf bacilli sera for the paratyphoid B bacillus and the calf bacilli and especially the results of the absorption tests are sufficient to differentiate the calf organism from the paratyphoid B bacillus.

*Comparison of the calf bacilli with the paratyphoid C bacilli.*

Table V shows that the paratyphoid C immune sera agglutinate certain of the calf bacilli, but only in relatively low dilutions.

The calf bacilli sera which exhibit very high titres for calf strains have practically no agglutinins for the paratyphoid C bacillus.

TABLE V.  
*Calf bacilli and bacilli of the para. C type.*

Serum.	AGGLUTINATION LIMITS FOR								
	Para. C. 1517.	Para. C. 1536.	Para. C. 782.	Para. C. 3414.	C. 3.	C. 4.	C. 5.	C. 10.	C. 4 Lawrence.
Bacillus para. C 1517.	2,500	2,500	2,500	1,600	250	..	250	..	..
Bacillus para. C 1536.	5,000	2,500	..	..	..	..	..	..	..
Bacillus para. C (Bagh).	6,400	6,400	..	..	800	200	800	200	1,600
Bacillus C. 3 .	50	..	50	50	125,000	83,000	..	..	125,000
Bacillus C. 4 .	250	..	..	..	..	..	..	..	..
Bacillus C. 5 .	50	1,000	50	50	..	..	50,000	..	50,000
Bacillus C. 10 .	250	250	..	..	..	..	..	..	..

No absorption tests were carried out in this series, but the results of the cross agglutination tests are sufficient to differentiate the calf bacillus from the paratyphoid C strain.

*Comparison of the calf bacilli with the guinea-pig bacilli.*

In table VI are shown the agglutinations of the calf and the guinea-pig bacilli by sera prepared by immunising rabbits with these strains. It will be seen that the guinea-pig bacillus sera agglutinate the guinea-pig and calf bacilli at the same titres, and the same holds for the calf bacilli sera.

TABLE VI.

*Cross agglutinations of calf bacilli and guinea-pig bacilli.*

Serum.	AGGLUTINATION LIMITS FOR							
	G. P. 2.	G. P. 4.	G. P. 24.	C. 3.	C. 4.	C. 5.	C. 6.	C. 4 Law- rence.
Bacillus G. P. 4 . . . .	..	50,000	50,000	..	..	..	50,000	50,000
Bacillus G. P. 24 . . . .	7,500	7,500	8,000	8,000	7,500	7,500	7,500	..
Bacillus C. 3 . . . . .	..	..	50,000	125,000	83,000	..	..	125,000
Bacillus C. 5 . . . . .	..	..	50,000	..	..	50,000	50,000	50,000
Bacillus C. 6 . . . . .	..	12,500	..	..	12,500	..	12,500	12,500

Table VII shows that the effect of absorbing a guinea-pig bacillus serum with the homologous bacillus is to remove the agglutinins both for the guinea-pig and calf bacillus. Similarly a calf bacillus serum absorbed with its own bacillus loses its agglutinins for both the calf and the guinea-pig strains.

TABLE VII.

*Absorption tests.*

Serum.	AGGLUTINATION LIMITS FOR	
	G. P. 24.	C. 3.
Bacillus G. P. 24 (original titre) . . . . .	8,000	8,000
Absorbed with bacillus G. P. 24 . . . . .	250	250
Bacillus C. 3 (original titre) . . . . .	50,000	125,000
Absorbed with bacillus C. 3 . . . . .	1,000	500



From the results of the above tests, we may conclude that the guinea-pig and the calf bacilli are identical species.

*Agglutination of the calf bacillus by Gaertner bacillus serum.*

Table VIII shows that the calf bacillus is agglutinated in low dilutions of the Gaertner serum.

TABLE VIII.

Bacillus.	SERUM B. GAERTNER DILUTION.				
	1-125	1-250	1-400	1-625	1-1250
C. 3 . . . . .	++	+	Trace	0	0
C. 5 . . . . .	++	+	Trace	0	0
Para. B. . . . .	..	0	0	0	0

++ = Definite sedimentation, the fluid remaining cloudy.

+ = Flocculation without sedimentation.

The serum used was about 2 years old and its original titre is noted as 1-1500, but we had no means of determining its present titre for the Gaertner bacillus.

*Summary of the results of agglutination and absorption tests.*

1. The calf bacillus can be differentiated from the paratyphoid B bacillus and from the paratyphoid C bacillus.
2. It is identical with the bacillus isolated from guinea-pig, dying of an epizootic disease in this laboratory.
3. It is probably an ærtrycke bacillus, but we have not excluded the possibility of its being a Gaertner bacillus. Its identity with the guinea-pig bacillus is in favour of its being an ærtrycke which is the commonest paratyphoid group organism associated with guinea-pig epizootics. Indirect evidence in support of its being B. ærtrycke is also afforded by the presence of co-agglutinin for the calf bacillus in paratyphoid B serum and of co-agglutinin for the paratyphoid B bacillus in the calf bacillus serum. It is known that the serum of an animal immunised with the ærtrycke bacillus usually contains co-agglutinin in considerable amount for the paratyphoid B bacillus and the converse is also true.

On the other hand, paratyphoid B serum has usually little or no co-agglutinin for Gaertner's bacillus and B. Gaertner serum, even when of high titre for Gaertner's bacillus, has only very slight action on the paratyphoid B bacillus.

5. AGGLUTINATION TESTS WITH SERA OF HEALTHY AND INFECTED CALVES AND WITH SERA OF DAMS OF HEALTHY AND INFECTED CALVES.

1. Sera obtained from apparently healthy calves.
2. Sera obtained from calves at the post-mortem.
3. Sera obtained from dams of healthy and infected calves.

Table IX gives the results obtained with the sera of apparently healthy calves. Four out of 10 sera tested, 40 per cent agglutinated one or more of the calf bacilli in dilutions varying from 1-25 to 1-250.

None of these sera agglutinated the paratyphoid B bacilli at 1-25 or upwards.

TABLE IX.

*Agglutination tests with the sera of healthy calves.*

Serum.	Age of calf.	AGGLUTINATION LIMITS FOR							
		C. 44.	C. 3.	C. 4.	C. 5.	C. 10.	C. 12.	C. 21.	Para. B.
C. 1	4 days	0	..	..	..	..	..	..	0
C. 2	3 weeks	0	0	0	0	0	..	..	0
C. 13	No record	125	..	..	125	50	..	..	0
C. 14	Do.	0	..	..	0	0	..	..	..
C. 15	2 months	0	..	..	0	0	..	..	..
C. 16	4 do.	0	..	..	0	0	0	0	..
C. 23	6 weeks	0	..	..	0	0	..	..	..
C. 24	3 do.	83	..	..	125	83	..	..	0
C. 26	3 do.	250	..	..	83	50	..	..	0
C. 27	No record	25	..	..	0	0	..	..	0

*Note.*—0 signifies no agglutination in dilutions of the serum from 1-25 upwards.

Table X shows that none of the sera obtained from calves dying in the acute stage of the disease and with intestinal ulceration contained agglutinins for the calf bacilli.

On the other hand, 4 out of 5 of the sera obtained from calves which showed no gross lesions at the post-mortem agglutinated one or more of the calf organisms. The bacillus was isolated from two of these calves.

TABLE X.

*Agglutination of calf bacilli by sera obtained from calves post-mortem.*

Serum.	Age.	Post-mortem.	Cultural result.	AGGLUTINATION LIMITS FOR											
				C. 4 Law- rence.	C. 3.	C. 4.	C. 5.	C. 6.	C. 8.	C. 9.	C. 10.	C. 11.	C. 12.	C. 25.	
C. 3	Days. 10-15	Intestinal le- sions.	Positive.	0	0	..	..	..	..	..	..	..	..	..	
C. 4	10-15	..	..	0	0	..	..	..	..	..	..	..	..	..	
C. 5	10-15	..	..	0	0	0	0	0	0	..	0	..	..	..	
C. 6	10-15	..	..	0	0	0	0	..	..	..	..	..	..	..	
C. 8	10-15	..	..	0	0	0	0	..	0	..	0	..	..	..	
C. 10	10	..	..	0	..	..	0	..	..	..	0	..	..	..	
C. 12	12	..	..	0	..	..	0	..	..	..	0	..	0	..	
C. 25	7	..	..	0	..	..	0	..	..	..	0	..	..	0	
C. 7	10	No lesions	Negative	50	0	..	..	..	..	..	..	..	..	..	
C. 9	10	..	Positive.	..	..	..	0	..	..	80	125	0	..	..	
C. 11	15	..	..	50	..	..	800	..	..	..	500	0	0	..	
	Months.														
C. 18	4	..	Negative	125	..	..	..	..	..	..	..	..	..	..	
C. 20	3	..	..	0	..	..	..	..	..	..	..	..	..	..	

Table XI gives the results obtained with the sera of 5 dams. Of these 3 were dams of infected calves and 2 of apparently healthy calves, whose sera, however, had been found to agglutinate the calf bacillus.

The sera of all 5 dams agglutinated one or more of the calf bacilli in low dilutions.

TABLE XI.

*Agglutination tests with sera of the dams of healthy and infected calves.*

SERUM.		AGGLUTINATION LIMITS FOR						
Dam No.	C.	C. 4 Lawrence.	C. 5.	C. 8.	C. 10.	C. 21.	C. 25.	C. 12.
44	27	0	..	..	83	..	..	..
360	25	0	..	0	..	..	50	..
371	21	50	..	0	..	83	..	..
475	12	50	50	0	..	..	..	25
607	24	0	..	0	83	..	..	..

#### 6. EXAMINATION OF THE BLOOD AND MILK OF THE DAMS OF HEALTHY AND INFECTED CALVES.

Blood cultures were made from the dams of 3 infected calves and 2 apparently healthy calves. All 5 bloods proved to be sterile.

Samples of milk were taken from the same dams and plated on MacConkey's medium directly and also after incubation in Taurocholate medium for some days. No organisms of the paratyphoid group were recovered from any of the samples.

#### 7. PATHOGENICITY OF THE CALF BACILLUS FOR ANIMALS.

Of 7 rabbits which were given an initial dose of .001 c.c. of a live 18-24 hours' old broth cultures of various calf bacilli intravenously, only one died—on the 7th day.

Two rabbits were given .01 c.c. of young broth cultures intravenously of these one died on the 7th day and one recovered. Both the rabbits which succumbed showed a localised inflammatory area in the diverticulum of the caecum, which was full of mucus. There was no ulceration of the mucous membrane. The rest of the intestine showed no gross changes.

One rabbit had purulent conjunctivitis and a congested friable spleen: the other, marked congestion of the base of one lung. The calf bacillus was recovered from the blood, bile, intestinal mucus, and organs of both animals.

*Guinea-pigs.*

Of 2 guinea-pigs which were given .005 c.c. of a young live broth culture of the calf bacilli under the skin, one showed only necroses of the skin at the site of inoculation; the other died on the 8th day.

*Post mortem.*—There was no local lesion, the spleen was very large and friable, the liver had recent lymph on the surface, the intestines showed no change. The bacillus was recovered from the blood, bile, and spleen.

*Calves.*

Two experiments were carried out in the calves which had been segregated at the dairy farm for some weeks before being sent to the laboratory.

*Calf No. 32.*—Age, about 7 weeks. Was given an intravenous injection of 4 c.c. of a 20 hours' old live broth culture of a recently isolated calf bacillus. Death took place in 2 hours.

*Post mortem.*—Lungs congested, a small oid abscess in the anterior upper surface of right lobe of the liver. No thrombosis of the jugular or other vein.

*Calf No. 33.*—Age, about 6-7 weeks. Injected intravenously with 0.6 c.c. of a live 22 hours' old broth culture of a recently isolated calf bacillus. Became ill on the 4th day. On the 5th day the calf was in a dying condition and was killed by chloroform.

*Post mortem.*—Lungs and heart, normal. Spleen somewhat enlarged and soft. Liver negative. Gall bladder full of clear bile. Intestines show no obvious lesions. Mesenteric glands moderately large, not congested or broken down. Kidneys; both have cystic pelves. The calf bacillus was recovered from the heart blood, bile, spleen, and mesenteric glands. The same bacillus was isolated from a stool passed on the previous day. The serum of the calf agglutinated the calf bacillus in a dilution of 1-125.

It will be seen that both rabbits, which died from the effects of intravenous inoculation with the live calf bacillus, showed local inflammatory lesions of the cæcum.

On the other hand, calf No. 33, which survived for five days after infection, did not show the intestinal ulceration so characteristic of the natural disease in young calves. This may have been due to the age of the calf, to the method of infection or the dose of the infecting

organisms : or again, it may be that the natural disease is due to a filter passer. (See below.)

8.    EXPERIMENTS TO DETERMINE WHETHER THE CALF DISEASE  
      IS DUE TO A FILTRABLE VIRUS.

On the analogy of hog-cholera and of the guinea-pig epizootics described by Bainbridge and O'Brien (both of which diseases, though associated with the presence of the artrycke bacillus, are attributed to a filter passing organism), it seemed possible that the calf disease might also be due to a filter passer. The 2 experiments given below are an attempt to obtain some evidence on this question.

*Experiment 1.*

The spleen of calf No. 29, which showed typical intestinal lesions and from the bile and spleen of which the artrycke-like bacillus was isolated, was emulsified in normal saline. The emulsion was well diluted and was filtered through a porous filter fitted to a filtration flask which was exhausted by a hand pump. Filtration was very slow ; it took several hours for 10 c.c. to come through. The filtrate was sterile. Two c.c. of the filtrate was injected into the ear veins of a rabbit. The rabbit showed no symptoms and was alive and healthy a month later. One c.c. of the filtrate was injected under the skin of a guinea-pig. The guinea-pig showed no signs of illness after the injection but was found dead 3 weeks later. Post mortem, no gross lesions were found.

*Experiment 2.*

The material used for filtration was the defibrinated blood of calf No. 32. This calf showed post mortem a congested patch in the caecum, no other lesions. The artrycke-like bacillus was isolated from the blood, bile, spleen, and lymph glands. The defibrinated blood was diluted 10 times and filtered as in the previous experiment. Filtration was again very slow. A sterile filtrate was obtained.

15 c.c. of the filtrate were given intravenously to a calf about 6 weeks old. The calf showed no symptoms and was quite healthy on the 15th day. (On the 15th day this calf was given an injection of a calf bacillus and died after the 5th day. Post-mortem examination showed no typical lesions of the intestine : see above.) We do not attach much importance to the negative results obtained in these two experiments.

The results of experiment No. 1 may probably be disregarded altogether as, on the analogy of hog-cholera, the filtrable virus of the calf

disease (if it exists) is probably pathogenic for calves only. Experiment No. 2 appears to furnish some evidence against the presence of a filtrable virus; however, as mentioned above, filtration was very slow, which would suggest that the bougie used (which was not marked) may have been less porous than a Chamberland F. or Berkefeld V.—the filters usually employed for this class of work.

#### 9. SUMMARY AND CONCLUSIONS.

The calf disease in its severe fatal form affects chiefly young calves. The characteristic lesion is ulceration and necrosis of the intestinal mucous membrane which is usually limited to the lower ileum, cæcum, and upper part of the colon.

2. The results of agglutination tests carried out with the sera of healthy calves and dams against the calf bacillus show that the infection was widespread among the cattle at the dairy farm and that many infected calves recovered.

3. The calf disease is associated with a bacillus of the paratyphoid Gaertner group which is present in the blood, bile, organs, mesenteric glands, and intestinal ulcers of calves which die of the disease.

4. The calf bacillus differs from the paratyphoid B and the paratyphoid C bacilli. It is probably an ærtrycke bacillus, but the experimental evidence obtained in this investigation does not exclude the possibility of its being Gaertner's bacillus.

5. The bacillus is pathogenic to rabbits and guinea-pigs and to calves when given intravenously.

6. The calf bacillus is probably not capable of causing an enteric-like fever in man, but, in certain circumstances, the ingestion of the flesh of infected animals might give rise to 'food-poisoning' outbreaks.

7. The question whether the calf disease is due to a filtrable virus with a secondary infection by the ærtrycke-like bacillus has not been decided. Further work is required to settle the question which is important from the point of view of prophylaxis. If the disease is due to a filter passer, not much protection can be expected from the employment of vaccines prepared with the calf bacilli.



# STUDIES IN THE VALUE OF THE WASSERMANN TEST.

No. I.

## FREQUENCY OF A POSITIVE WASSERMANN REACTION IN AN UNSELECTED ADULT MALE INDIAN POPULATION.

BY

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IN studying the literature regarding the value of a positive Wassermann reaction in certain diseases other than syphilis, one is struck with the fact that no importance has been paid to the possibility of a certain proportion of these positive findings being due to latent syphilis in the patient and not to the disease under investigation.

This investigation was undertaken in order to estimate the percentage of positive Wassermann reactions obtained in an unselected and apparently healthy male Indian population. By the kindness of Major McKendrick, I.M.S., Director, Pasteur Institute of India, Kasauli, I have been able to examine the sera of 400 totally unselected Indian male patients, between the ages of 20 and 60 years, who attended the Pasteur Institute for antirabic treatment. These patients were from the various parts of the Punjab, Baroda, Behar, Bengal, Bombay, Gujerat, and North-Western Provinces. Tables I and II clearly indicate the class constitution and unselected character of this population. It will here be seen that they are not drawn from any one caste or religion and that rich and poor are included at random. The result shows that a positive Wassermann reaction was demonstrable in 88 out of the 400 cases tested, which is equivalent to 22 per cent. I have included in the *negative* results 9 cases whose sera only partially deviated 3 minimal hæmolytic

doses of complement. Table III shows the age constitution of the population examined.

The sera of 30 healthy individuals, selected in the first instance because of an emphatically negative syphilitic history and because of no probability of such infection, were examined and each and all gave a completely negative reaction.

The technique employed throughout this investigation is essentially the same as 'Method Number Four' described in the Medical Research Committee Report (1918). The main differences from that method were (1) that an additional tube containing 8 minimal hæmolytic doses of complement was included, and (2) an additional control in the shape of a weakly syphilitic serum which deviated 3 minimal hæmolytic doses of complement was used as well as a strongly syphilitic serum deviating 8 minimal hæmolytic doses of complement. An objection might be raised that the antirabic treatment in itself might invalidate the results obtained, and therefore I examined the sera of 28 cases before starting treatment, of 23 cases early in treatment, and of all these cases after the termination of treatment. In no case was I able to demonstrate any alteration of the Wassermann reaction as a result of treatment.

The importance of this investigation lies in the fact that comparative research may be instituted into the possibility of the occurrence of the Wassermann reaction for syphilis in other affections or under special circumstances, and that such research is almost valueless without a basis of comparison such as is presented here.

TABLE I.

*Showing religion and caste constitution of the population examined.*

	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
<b>HINDU.</b>							
Ahir . . . . .	6	3	2	1	6	6	12
Arora . . . . .	2	0	0	0	0	2	2
Baniya . . . . .	12	2	0	2	4	12	16

TABLE I.—(Contd.)

	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
<i>HINDU—contd.</i>							
Bhar . . . . .	2	0	0	0	0	2	2
Bhat . . . . .	1	1	0	0	1	1	2
Bhatiya . . . . .	2	0	0	0	0	2	2
Bhisti . . . . .	2	0	0	0	0	2	2
Brahmin . . . . .	41	4	4	5	13	41	54
Chamar . . . . .	10	0	3	1	4	10	14
Chhimba . . . . .	2	0	0	0	0	2	2
Deshmali . . . . .	3	0	0	0	0	3	3
Dhimar . . . . .	2	0	1	1	2	2	4
Dhobi . . . . .	2	0	0	0	0	2	2
Dosadh . . . . .	3	0	1	0	1	3	4
Gadariya . . . . .	0	1	0	0	1	0	1
Gola . . . . .	3	0	0	0	0	3	3
Gond . . . . .	0	1	0	0	1	0	1
Gujar . . . . .	5	0	0	0	0	5	5
Gurkha . . . . .	0	1	0	0	1	0	1
Hajjam . . . . .	1	0	1	0	1	1	2
Kahar . . . . .	7	1	1	0	2	7	9
Kalwar . . . . .	3	0	0	0	0	3	3
Kanadi . . . . .	2	0	0	0	0	2	2
Kansari . . . . .	1	2	0	0	2	1	3
Kayasth . . . . .	6	1	2	1	4	6	10
Khosta . . . . .	2	0	0	1	1	2	3
Koiri . . . . .	2	0	0	0	0	2	2
Koli . . . . .	4	1	0	0	1	4	5

TABLE I.—(Contd.)

	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
HINDU—concl'd.							
Kumhar . . . . .	1	0	0	0	0	1	1
Kurmi . . . . .	9	0	0	1	1	9	10
Mahar . . . . .	1	0	0	2	2	1	3
Mali . . . . .	1	0	0	0	0	1	1
Maratha . . . . .	8	0	1	1	2	8	10
Mehtar . . . . .	14	0	2	1	3	14	17
Mochi . . . . .	0	0	1	0	1	0	1
Jat . . . . .	13	0	1	0	1	13	14
Lod . . . . .	1	0	0	0	0	1	1
Lohar . . . . .	2	0	0	0	0	2	2
Pasi . . . . .	0	1	0	0	1	0	1
Patvekari . . . . .	0	1	0	0	1	0	1
Rajput . . . . .	15	2	0	0	2	15	17
Ramoshi . . . . .	2	0	0	0	0	2	2
Rao . . . . .	1	1	0	0	1	1	2
Sadhu . . . . .	2	0	0	0	0	2	2
Sonar . . . . .	3	0	1	0	1	3	4
Teli . . . . .	0	0	1	0	1	0	1
Turkhan . . . . .	3	0	0	0	0	3	3
Vadda . . . . .	1	0	0	0	0	1	1
MUSSALMAN.							
Awan . . . . .	2	0	0	0	0	2	2
Fakir . . . . .	3	0	0	0	0	3	3
Jolaha . . . . .	2	0	0	0	0	2	2
Moghul . . . . .	2	0	0	0	0	2	2

TABLE I.—(Concl'd.)

	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
<b>MUSSALMAN.—concl'd.</b>							
Pathan . . . . .	24	6	0	2	8	24	32
Rajput . . . . .	6	0	1	0	1	6	7
Saiyid . . . . .	14	2	0	2	4	14	18
Sheikh . . . . .	21	4	0	3	7	21	28
<b>SIKH.</b>							
Jat . . . . .	27	0	3	3	6	27	33
<b>INDIAN CHRISTIAN.</b>							
Protestant . . . . .	5	0	0	0	0	5	5
Roman Catholic . . . . .	3	0	0	0	0	3	3

*Summary of Table I.*

Religion.	Number of cases examined by the Wassermann test.	RESULTS.	
		Positive.	Negative.
Hindus . . . . .	265	62	203
Mussalmans . . . . .	94	20	74
Sikhs . . . . .	33	6	27
Indian Christian . . . . .	8	0	8
<b>TOTAL . . . . .</b>	<b>400</b>	<b>88</b>	<b>312</b>

The percentage of positive results in the total of 400 cases was thus 22 per cent.

TABLE II.

*Showing occupation of the population examined.*

OCCUPATION.	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive	Nega- tive.	Total.
<b>I.—AGRICULTURE.</b>							
(1) Landowners . . .	40	3	2	2	7	40	47
(2) Landowner and cultivators .	49	8	4	2	14	49	63
(3) Labourers . . .	18	3	2	2	7	18	25
<b>II.—INDUSTRY.</b>							
(1) Cotton spinners . . .	4	1	0	0	1	4	5
(2) Jute weavers . . .	2	1	0	0	1	2	3
(3) Tanners . . .	1	0	0	0	0	1	1
(4) Makers of leather articles .	3	0	1	1	2	3	5
(5) Workers in brass . . .	2	1	0	0	1	2	3
(6) Goldsmiths . . .	3	0	1	0	1	3	4
(7) Potters . . .	3	1	1	1	3	3	6
(8) Carpenters . . .	6	1	0	0	1	6	7
(9) Sweetmeat maker . . .	2	0	1	0	1	2	3
(10) Washermen . . .	2	0	0	0	0	2	2
(11) Barbers . . .	2	1	0	0	1	2	3
(12) Sweepers . . .	10	0	0	2	2	10	12
<b>III.—TRADE.</b>							
(1) Money-lenders . . .	2	1	0	0	1	2	3
(2) Traders in piece-goods . .	2	1	0	0	1	2	3
(3) Traders in metals . . .	4	0	0	2	2	4	6
(4) Traders in pottery . . .	6	1	1	0	2	6	8
(5) Sellers of milk . . .	6	0	2	0	2	6	8
(6) Sellers of sweetmeats . .	2	0	0	0	0	2	2

TABLE II.—(Contd.)

OCCUPATION.	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
IV.—ARMY.							
(1) Havildars . . . .	2	0	0	0	0	2	2
(2) Duffedars . . . .	1	0	0	0	0	1	1
(3) Sepoys . . . .	31	1	1	1	3	31	34
V.—POLICE.							
(1) Sub-Inspectors . . .	1	0	0	0	0	1	1
(2) Head Constables . .	2	0	0	0	0	2	2
(3) Constables . . . .	12	3	1	1	5	12	17
VI.—PUBLIC ADMINISTRATION.							
(1) Clerks . . . .	16	2	1	1	4	16	20
(2) Peons . . . .	4	1	0	0	1	4	5
VII.—POST OFFICE.							
(1) Clerks . . . .	2	0	0	1	1	2	3
(2) Peons . . . .	6	0	1	1	2	6	8
VIII.—RAILWAYS.							
(1) Station Masters . . .	3	0	0	0	0	3	3
(2) Assistant Station Masters .	2	0	1	0	1	2	3
(3) Engine Drivers . . .	4	1	0	0	1	4	5
(4) Coolies . . . .	17	3	1	5	9	17	26
IX.—IRRIGATION.							
(1) Overseers . . . .	1	0	0	0	0	1	1
(2) Sub-Overseers . . .	1	0	0	0	0	1	1
(3) Contractors . . . .	1	0	0	1	1	1	2
(4) Coolies . . . .	2	0	0	0	0	2	2



TABLE II.—(Concl'd.)

OCCUPATION.	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
X.—RELIGION.							
(1) Priests . . . . .	2	0	2	1	3	2	5
XI.—LAW.							
(1) Lawyer's clerks . . . .	6	1	1	0	2	6	8
XII.—MEDICINE.							
(1) Assistant Surgeon . . .	1	0	0	0	0	1	1
XIII.—EDUCATION.							
(1) School Masters . . . . .	3	0	0	0	0	3	3
(2) Students . . . . .	5	0	0	0	0	5	5
XIV.—DOMESTIC SERVICE.							
(1) Cooks . . . . .	4	0	2	0	2	4	6
(2) Bearers . . . . .	10	0	0	3	3	10	13
(3) Bhistis . . . . .	2	0	0	0	0	2	2
(4) Syces . . . . .	2	0	0	0	0	2	2

TABLE III.

*Showing the age distribution of the population examined.*

Age periods.	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
20-30 . . . . .	146	15	14	8	37	146	183
30-40 . . . . .	89	6	7	10	23	89	112
40-50 . . . . .	44	8	2	7	17	44	61
50-60 . . . . .	33	6	3	2	11	33	44

CONCLUSIONS.

1. Twenty-two per cent of an apparently healthy Indian male adult population, taken without selection, showed latent or clinically inactive syphilis as demonstrated by the Wassermann test.

2. There is no reason to suppose that this population differed appreciably from a random population of the same class and age constitution. The class constitution was a very varied one.

3. The percentage of positive Wassermann reactions amongst the various castes and religions, does not indicate that any one caste or religion is more syphilitic than another.

I wish to express my indebtedness to Lieut.-Colonel W. F. Harvey, I.M.S., Director, Central Research Institute, Kasauli, for much helpful advice and criticism.

REFERENCE.

Medical Research Committee Report (1918) No. 14. pp. 36-41

# STUDIES IN THE VALUE OF THE WASSERMANN TEST.

## No. II.

### SIGNIFICANCE AND VALUE OF A POSITIVE WASSERMANN REACTION IN LEPROSY.

BY

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A NUMBER of authors have investigated the existence of a positive Wassermann reaction in lepers. Sutherland and Mitra (1915) give a résumé of the findings of different observers and state that they themselves found that 4 cases out of 14 individuals affected with the anæsthetic form of leprosy, and 7 out of 20 affected with other forms, gave a positive Wassermann reaction.

I have examined the sera of 100 cases of undoubted leprosy, all males between the ages of 20 and 60 years, and these are shown in Table I according to caste and religion, and in Table II according to type of disease. Of these 100 cases, 34 were of the nodular, 52 of the anæsthetic, and 14 of the mixed form, and the number of positive Wassermann reactions were for these several types 17, 16, and 8, respectively, which is 41 per cent.

Besides these cases, sera of 12 children born of leper parents were examined. The ages of these children were from 11 months to 15 years, and they all gave a negative reaction. At present they show no signs of leprosy and are healthy.

The following is the history of these 12 children :—

- (1) *Six children*.—These were the children of a leper father, 4 by the first wife and 2 by the second, the youngest being 11

months old and the eldest 15 years. The father of these children says that the first wife died of leprosy. The blood of the father and of the second wife, both lepers, gave a strongly positive Wassermann reaction—sera completely deviating 8 minimal hæmolytic doses of complement. All the six children are strong and healthy. There was no history of abortion or miscarriage either in the first or second wife and the father denies totally any specific infection.

(2) *Two children.*—4 years and 8 years of age. The mother of these children, a leper, gives a strongly positive Wassermann reaction, but the father, also a leper, gives a negative reaction. They have had eight children, one died of leprosy when 17 years of age, the others are alive with no evidence of leprosy or any other disease. There was no history of abortion or miscarriage in the mother.

(3) *Four children.*—3, 8, 11, and 13 years of age. These were children of undoubted leper parents and were all strong and healthy. The parents' blood was not examined in the case of the children of 11 and 13 years of age, but the father of the other two (3 and 8 years) gave a strongly positive Wassermann reaction.

The technique employed throughout this investigation is the same as 'Method Number Four' described by the Medical Research Committee Report (1918), the only difference being the inclusion of further controls as described in my paper (Study No. 1, 1919).

The fact of complete immunity of children of lepers to the disease is well known, at all events up to certain ages. It is also known that continued association of children with leper parents may result in contraction of the infection. The small number of cases of children with which I have illustrated this paper is interesting as showing the same immunity. The absence of a positive Wassermann reaction in these children, which to a large extent is found in those suffering from leprosy, constitutes additional evidence for the view that this positive reaction is characteristic of the disease and not due to underlying syphilitic infection. Moreover, the fact of these leper parents having healthy children whilst giving a strongly positive Wassermann reaction, is evidence against the reaction being syphilitic.

TABLE I.

*Showing religion and caste constitution of the leper population examined.*

	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
<b>HINDU.</b>							
Brahmin . . . . .	5	0	0	1	1	5	6
Chamar . . . . .	1	0	0	1	1	1	2
Chasa . . . . .	2	0	1	0	1	2	3
Chattri . . . . .	4	0	0	1	1	4	5
Dhobi . . . . .	2	0	0	0	0	2	2
Dosad . . . . .	1	0	1	0	1	1	2
Gola . . . . .	1	1	0	3	4	1	5
Jolaha . . . . .	2	0	0	0	0	2	2
Kahar . . . . .	0	2	0	1	2	0	3
Kayasth . . . . .	10	0	0	2	2	10	12
Kaibarta . . . . .	3	0	0	0	0	3	3
Kewat . . . . .	0	0	0	1	1	0	1
Koli . . . . .	2	0	0	1	1	2	3
Kurmi . . . . .	2	0	0	0	0	2	2
Lohar . . . . .	0	1	0	0	1	0	1
Mochi . . . . .	1	0	0	1	1	1	2
Rajput . . . . .	1	0	0	1	1	1	2
Teli . . . . .	1	0	0	1	1	1	2
Tamuli . . . . .	1	1	0	0	1	1	2
Turkhan . . . . .	1	0	0	1	1	1	2
<b>MUSSALMAN.</b>							
Saiyyid . . . . .	4	1	0	0	1	4	5
Sheikh . . . . .	3	1	1	2	4	3	7

TABLE I.—(Contd.)

	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
ANGLO-INDIAN.							
Protestant . . . . .	0	1	0	1	2	0	2
Roman Catholic . . . . .	4	1	2	0	3	4	7
Jew . . . . .	1	0	0	0	0	1	1
INDIAN CHRISTIAN.							
Protestant . . . . .	6	0	0	9	9	6	15
Roman Catholic . . . . .	1	0	0	0	0	1	1

TABLE II.

*Showing the type of disease of the leper population examined.*

TYPE OF DISEASE.	NUMBER OF DOSES OF COMPLEMENT DEVIATED IN THE WASSERMANN REACTION.				ASSEMBLED RESULTS. WASSERMANN REACTION.		
	0	3	5	8	Posi- tive.	Nega- tive.	Total.
Nodular . . . . .	17	5	2	10	17	17	34
Anæsthetic . . . . .	36	2	3	11	16	36	52
Mixed . . . . .	6	2	0	6	8	6	14

The percentage of positive results in the total of 100 cases was thus 41 per cent.

## CONCLUSIONS.

(1) In view of the fact that an investigation (Study No. 1, 1919) gave a positive Wassermann reaction of 22 per cent for an unselected Indian male adult population, I infer that such a proportion as 41 per cent occurring in an unselected adult male leper population must have the significance generally given to it that it is a sign of leprosy and not merely of syphilis.

(2) Further evidence of the characteristic nature of the Wassermann reaction in leprosy is afforded by the fact that lepers who had been able to reproduce children, healthy both as regards the disease from which they were suffering and as regards any evidence of syphilis, themselves gave strongly positive Wassermann reaction.

I wish to express my thanks to Lieut.-Colonel W. F. Harvey, I.M.S., Director, Central Research Institute, Kasauli, for advice and criticism, and also to the Rev. J. Ryburn, of Subathu, Punjab, and to Major N. P. Sinha, I.M.S., Police Surgeon, Calcutta, for placing facilities at my disposal during this investigation.

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# THE PREVALENCE OF ANKYLOSTOMIASIS IN THE MADRAS PRESIDENCY.

BY

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## INTRODUCTION.

THIS report is a continuation of my previous Ad Interim Report<sup>(1)</sup>. The inquiry carried out among the coolies collected at the Negapatam Emigration Dépôt showed that the percentage of hookworm infection is 98.4 per cent for the rural population of the southern half of the Madras Presidency and about 91 per cent for the urban population of Negapatam town. The infection is found in all classes irrespective of caste, age, sex, occupation, and social status.

In the course of this new investigation, each case was taken up as it came. After a microscopic examination of a fecal sample, the person, if found clinically fit, was given an anthelmintic treatment under hospital conditions. His stools were collected and examined during the five days which followed treatment or until no hookworms were found on two consecutive days. A note was kept of all symptoms and after-effects. Second and subsequent treatments were administered at intervals of 10 days or more, and, as far as could be, each case was followed up until cure was established. I mean by 'cure' the absence of hookworms in stools after an extra treatment, or the absence of their ova 12 or more days after a treatment.

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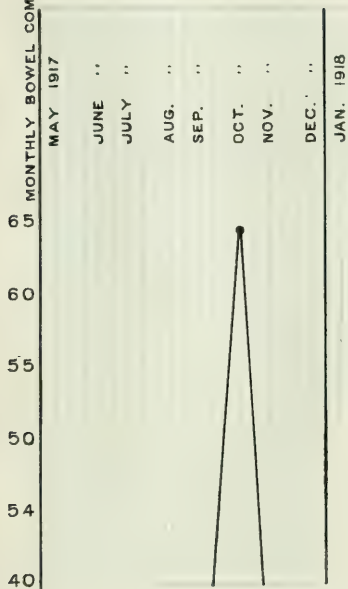
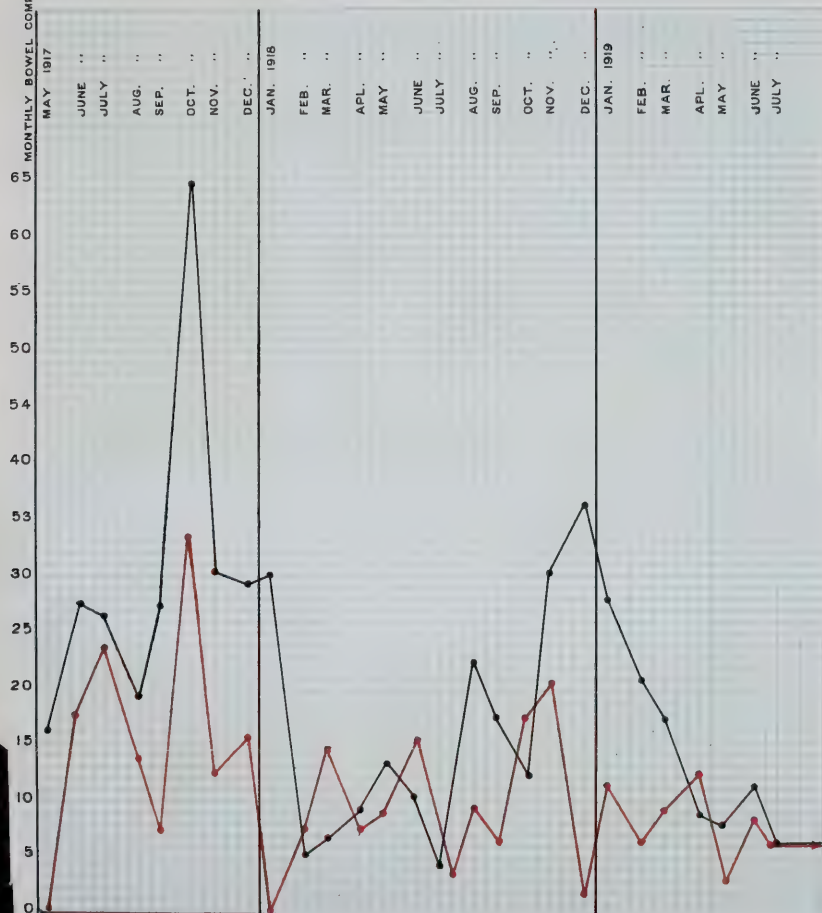


CHART I.—Monthly bowel complaints  
rate in the untreated and treated  
for hookworm.

Untreated for hookworm. —●—  
Treated for hookworm. —●—



For the microscopic examination of fæces Howard's method was adopted throughout. The technique has been fully described by Clayton Lane.<sup>(2)</sup>

The method of treatment adopted was the well-known routine method. The person is given a light diet between 4 and 5 P.M. followed by Epsom salts at 8 P.M. The next morning the anthelmintic is dispensed in three portions at hourly intervals, provided the previous night purge has acted well. Epsom salts are given two hours after the ingestion of the anthelmintic, and a light diet is allowed after the purgative has acted once. The anthelmintics used were thymol and chenopodium oil. The standard adult dose of 60 grains and 48 minims, respectively, was administered unless otherwise contra-indicated. Thymol was finely powdered with its own weight of sugar, and oil of chenopodium was given either on a lump of sugar or in a mucilage of acacia emulsion prepared on the spot. As 48 minims chenopodium oil was found to be more toxic than 60 grains thymol, I gave preference to this drug and treated most of my cases with it. None of the cases figuring in this report had previously been treated for hookworm.

When fæces are to be examined for worms, they are shaken with a large amount of water and thrown over a fine wire gauze sieve of 40 meshes to an inch. The solid material is then transferred from the sieve to black-bottomed Petri dishes, when the worms are separated, classified and counted.

Hæmometric estimations were carried out with a view to determine how far the percentage of hookworm infection may modify the hæmoglobin content and affect the eosinophiles. Only persons who are proved to be free from tuberculosis, malaria, kala-azar or similar infecticous diseases were examined. For the purpose of determining the hæmoglobin percentage, we converted the hæmoglobin into a dilute solution of acid hæmatin which was then matched with a solution of acid hæmatin contained in a graduated wedge-shaped cell. For the eosinophile count the blood film was stained with Giemsa, two hundred corpuscles were counted, one hundred near the edges and another hundred in the body of the film, and the mean was taken.

This report covering a period of twenty-seven months, from 9th April, 1917, to 31st July, 1919, chiefly deals with the percentage and intensity of the hookworm infection.

It was considered advisable to have small controlled communities for observation over a prolonged period; and so, with the kind permission

of the Inspector-General of Prisons, the convicts of Trichinopoly and Coimbatore Jails were selected: and on the advice of the Surgeon-General, Dindigul Town and a few neighbouring villages were chosen as suitable places to study how far preventive measures could be introduced in the free urban and rural communities of this Presidency.

### I. TRICHINOPOLY JAIL.

The Trichinopoly Jail has a daily average convict population of 1,114. It is divided into eleven blocks, each with its sanitary latrine accommodation. The night-soil is carried away in covered receptacles and is trenched close to the jail wall. The fly nuisance is considerable. The water-supply was received (up to October 1917) by pipes from the town water-works and carted to the blocks in barrels. At present it is led directly by pipes.

The convicts mostly hail from the districts of Tanjore, Trichinopoly, Madura, Rammad, and Tinnevely. Those who were treated for hookworm were adult Tamilian males of ages varying from 20 to 60 years, and were of average constitution and health. They thus favourably compare in most respects with the coolies examined at the Negapatam Emigration Depot.

A new factor has, no doubt, been introduced in the life of the convict on his being admitted into the jail, for his chances of infection from polluted soil or from flies are evidently less. Freedom from soil infection is almost certain, as the latrines are sanitary and well kept. The fly nuisance is considerable it is true, but, nevertheless, comes short of the same evil in most towns and villages. Further, the kitchen is fly-proof and the danger of food infection is little. However much this new factor may tell on the percentage and intensity of the hookworm infection, it is highly improbable that its effects could be felt within a week from the time of the prisoner's admission into the jail; and so the hookworm contents of the hosts, determined within that period, ought to represent the percentage and intensity of the infection in the respective districts they come from.

The percentage of hookworm infection per district, as determined by the microscopic examination of faecal samples from convicts whose stay in jail did not exceed five days, is recorded in Table I, and is a confirmation of my Negapatam work.

TABLE I.

*Microscopic examination (before treatment) of fecal samples taken within 5 days of admission to Jail—by districts.*

Districts.	TRICHINOPOLY JAIL CASES.			NEGAPATAM DEPÔT CASES.
	Total cases examined.	Cases showing hookworm ova.	Percentage showing hookworm ova.	Percentage showing hookworm ova.
1. Tanjore . . . .	302	299	99.1	99.3
2. Trichinopoly . . .	173	168	97.3	98.4
3. Madura . . . .	93	84	90.6	97.6
4. Ramnad . . . .	20	19	95.0	98.0
5. Tinnevelly . . . .	17	16	94.1	95.0

The percentage of infection for the districts of Tanjore, Trichinopoly, Madura, and Ramnad is also corroborated by the results obtained from the macroscopic examination after treatment (Table II). Further investigation is necessary for the Tinnevelly District.

TABLE II.

*Macroscopic examination (after treatment) of feces to show the percentage of hookworm infection by districts : period of stay in Jail—0 to 5 days.*

Districts.	Total cases treated.	TOTAL CASES INFECTED WITH HOOKWORMS.			PERCENTAGES SHOWING HOOKWORMS.		
		A. duodenale infection, single or combined.	Necator infection, single or combined.	A. duodenale and Necator infection, single and combined.	A. duodenale infection, single or combined.	Necator infection, single or combined.	A. duodenale and Necator infection, single and combined.
1. Tanjore . . .	122	97	122	122	79.5	100.0	100.0
2. Trichinopoly .	88	39	87	87	44.3	98.9	98.9
3. Madura . . .	44	5	40	40	11.3	90.9	90.9
4. Ramnad . . .	38	14	36	36	30.8	94.7	94.7
5. Tinnevelly . .	6	1	6	6	..	..	..

Table II also shows that the hookworm infection of the Tamil districts is a *Necator* infection. The *A. duodenale* infection is relatively low and varies widely in the different districts. These conclusions find a further support from a study of the intensity of the infection.

The intensity of the hookworm infection is calculated from the total hookworm content. By total hookworm content is meant the sum total of hookworms removed from the host during treatment until 'cure' has been established as shown by the absence of hookworms and ova from the stools. As the first treatments removed 90 to 95 per cent of hookworms, the 5 to 10 per cent balance passed on subsequent treatments is not likely to be due to re-infection, and it therefore appears in the total under hookworms removed on the day of first treatment.

TABLE III.

*Intensity of hookworm infections by districts (macroscopic examination for worms after treatment) : period of stay in Jail—0 to 5 days.*

Districts.	Total cases treated.	TOTAL HOOKWORMS REMOVED.			AVERAGE NUMBER OF HOOKWORMS PER CASE TREATED.			Percentage of <i>A. duodenale</i> to total hookworms.
		<i>A. duodenale</i> .	<i>Necator</i> .	<i>A. duodenale</i> and <i>Necator</i> .	<i>A. duodenale</i> .	<i>Necator</i> .	<i>A. duodenale</i> and <i>Necator</i> .	
1. Tanjore .	115	770	10,642	11,412	6.7	92.5	99.2	6.7
2. Trichinopoly .	88	166	3,555	3,721	1.9	40.4	42.3	4.4
3. Madura .	44	13	1,294	1,307	0.2	29.4	29.7	1.0
4. Ramnad .	38	39	904	943	1.0	23.8	24.8	4.1
5. Tinnevelly .	6	5	109	114	0.8	18.1	18.9	4.3

As may be gathered from Table III, the district of Tanjore is most heavily infected with an average of 99 hookworms per person; Madura, Ramnad, and Tinnevely are in descending order. The relative exemption of the Madura District from *A. duodenale* infection is striking.

Table III (last column) also supports the conclusion drawn from Table II that the hookworm infection in these parts is mainly a *Necator* infection.



The male and female *Necators* in a series of 150 cases were present in almost equal numbers; the *A. duodenale* males were in the proportion of 5 to 4 females. *Necator* males varied in length between 5 and 12.5 mm. and the females between 7.5 and 18 mm.; the *A. duodenale* males were from 7 to 14 mm. long and the females from 8.5 to 17 mm.

*A. ceylanicum*, which figures to the extent of 9.5 per cent in Clayton Lane's<sup>(3)</sup> Berhampore Jail series of 150 convicts, has not so far been detected here in Trichinopoly. Twenty-four recently killed dogs were post-mortemed and all were found infected with *A. caninum* while only five harboured *A. ceylanicum*. The average intensity of infection was 29 hookworms per dog.

Of the flies caught near the jail kitchen, 3 per cent were found to harbour hookworm ova or their larvæ.

The microscopic examination for ova other than those of hookworms was never made exhaustive and, therefore, the percentage of infection with these helminths, as noted in Table IV, is somewhat underestimated. The figures show, however, that the infection actually present in convicts from the five districts is not lower than is indicated by the figures.

TABLE IV.

*Percentage of helminthic infections, other than hookworm, discovered within 5 days of admission to Jail (microscopic examination of faecal samples before treatment).*

Helminths.	Cases showing ova.	Percentage showing ova.
1. <i>Ascaris lumbricoides</i> . . . . .	304	49.9
2. <i>Trichiuris trichiura</i> . . . . .	232	38.3
3. <i>Strongyloides</i> . . . . .	38	6.4
4. <i>Oxyuris vermicularis</i> . . . . .	14	2.3
5. <i>Tenia solium</i> . . . . .	1	0.1
6. <i>Tenia saginata</i> . . . . .	1	0.1
Total cases examined . . . . .	605	

The frequency of plural infections is shown in Table V. The hookworms may be found associated with every other parasite.

TABLE V.

*Frequency of plural helminthic infections (including hookworm) discovered by the microscope within 5 days of admission to Jail.*

Frequency of distribution.		Total cases showing ova.	Percentage showing ova.	
Single infections.	{ Ankylostomes . . . . .	140	142	23.4
	{ Ascaris lumbricoides . . . . .	1		
	{ Trichiuris trichiura . . . . .	1		
Double infections.	{ Ankylostomes + Ascaris. . . . .	184	319	52.7
	{ " + Trichiuris . . . . .	115		
	{ " + Strongyloides . . . . .	17		
	{ " + Tænia sol. . . . .	1		
	{ " + Tænia sag. . . . .	1		
{ Ascaris + Trichiuris . . . . .	1			
Triple infections.	{ Ankylostomes + Ascaris + Trichiuris . . . . .	93	116	19.1
	{ " + " + Strongyloides . . . . .	9		
	{ " + " + Oxyuris . . . . .	4		
	{ " + Trichiuris + Strongyloides . . . . .	4		
	{ " + " + Oxyuris . . . . .	6		
Quadruple infections.	{ Ankylostomes + Ascaris + Trichiuris + Strongyloides . . . . .	8	1	1.9
	{ Ankylostomes + Ascaris + Trichiuris + Oxyuris . . . . .	4		
Total cases examined . . . . .		= 605		

*Oxyuris vermicularis* ova are rarely seen under the microscope. The adult worms were found in 99 per cent of a series of 1,700 convicts treated. The universality of this infection gives a fair idea of the insidious individual habits of the people of these districts.

Of the 24 dogs post-mortemed, only one was infected with roundworms. The flies examined at the jail occasionally had roundworm and whipworm ova in their intestinal contents.

Coprophagia, indicating the extent to which food is liable to faecal contamination, is found to be 74.2 per cent.

In Table VI the cases are distributed according to the various hæmoglobin ranges. The average hæmoglobin per case is found to be 74.6 per cent.

TABLE VI.

*Frequency of distribution of hæmoglobin percentage (before treatment) in cases examined at Trichinopoly Jail.*

Hæmoglobin percentage.	Total cases.	Percentage to total cases.
30-40 per cent . . . . .	1	<b>0·3</b>
40-50 „ . . . . .	6	<b>1·9</b>
50-60 „ . . . . .	20	<b>6·3</b>
60-70 „ . . . . .	74	<b>23·5</b>
70-80 „ . . . . .	115	<b>37·2</b>
80-90 „ . . . . .	88	<b>27·8</b>
90-100 „ . . . . .	11	<b>3·4</b>
Total cases examined . . . . .	=315	Average hæmoglobin percentage = <b>74·6</b>

A similar examination of 300 convicts in Coimbatore Jail gave an average hæmoglobin percentage of 72·4.

TABLE VII.

*Frequency of distribution of hæmoglobin percentage (before treatment) in cases examined at Coimbatore Jail.*

Hæmoglobin percentage.	Total cases.	Percentage to total cases.
40-50 per cent . . . . .	2	<b>0·6</b>
50-60 „ . . . . .	16	<b>5·3</b>
60-70 „ . . . . .	65	<b>21·6</b>
70-80 „ . . . . .	123	<b>41·0</b>
80-90 „ . . . . .	86	<b>28·6</b>
90-100 „ . . . . .	8	<b>2·6</b>
Total cases examined . . . . .	=300	Average hæmoglobin percentage = <b>72·4</b>

The investigations at Negapatam had shown that the percentage of eosinophiles in 500 boys of school-going age was 10·7 per cent. As *Ascaris* and *Trichiuris* infections were very often found there associated with the hookworm infection, it was not possible to determine accurately whether this marked eosinophilia was due to the hookworms alone. 213 hookworm cases free from *Ascaris* and *Trichiuris* were examined at the jail for eosinophilia and the percentage was found to be 10·2 (Table VIII). But as the cases harboured *Oxyuris* as well the same doubt still subsists.

TABLE VIII.

*Frequency of distribution of eosinophilia percentage (before treatment) in cases examined at Trichinopoly Jail.*

Eosinophilia percentage.	Total cases.	Percentage to total cases.
0-3 per cent . . . . .	2	0·9
3-5     " . . . . .	10	4·7
5-10    " . . . . .	105	49·3
10-15   " . . . . .	77	36·1
15-20   " . . . . .	16	7·5
20-24   " . . . . .	3	1·4
Total cases examined . . .	= 213	Average eosinophilia percentage = 10·2

That hookworm infection may be a predisposing cause in medical diseases, could only be observed here in bowel complaints: diarrhoea, dysentery, etc. The mille rate of infection for the two groups of treated and untreated for hookworm is given in Table IX, and is shown graphically in Chart 1. It is evident that the riddance from hookworm infection confers some protection to the convicts.

TABLE IX.

*Average monthly population (Trichinopoly Jail), and the bowel complaints rate in 'treated' and 'untreated' for hookworm.*

Months.	TREATED FOR HOOKWORM.			UNTREATED FOR HOOKWORM.		
	Average monthly population.	Number of 'bowel complaints.'	Monthly per mille rate.	Average monthly population.	Number of 'bowel complaints.'	Monthly per mille rate.
1917.						
May . . .	47	0	0·0	1,236	20	16·1
June . . .	117	2	17·1	1,081	30	27·7
July . . .	213	5	23·5	848	23	27·1
August . . .	299	4	13·4	780	15	19·2
September . . .	420	3	7·1	696	19	27·3
October . . .	421	14	33·2	680	44	64·7
November . . .	399	5	12·5	654	20	30·6
December . . .	381	6	15·7	676	16	23·6
1918.						
January . . .	441	0	0·0	592	13	21·9
February . . .	514	4	7·7	552	3	5·4
March . . .	539	8	14·8	542	3	5·5
April . . .	563	4	7·1	527	5	9·4
May . . .	549	5	9·1	531	7	13·1
June . . .	568	9	15·8	468	5	10·6
July . . .	573	2	3·4	439	2	4·2
August . . .	618	6	9·7	397	9	22·6
September . . .	629	4	6·3	448	8	17·8
October . . .	585	10	17·1	636	8	12·5
November . . .	533	11	20·6	764	2	30·1
December . . .	521	1	1·9	719	2	36·1
1919.						
January . . .	608	7	11·5	717	20	27·8
February . . .	634	4	6·4	816	17	20·8
March . . .	646	6	9·3	801	14	17·4
April . . .	656	8	12·2	784	7	8·9
May . . .	664	2	3·0	769	7	8·1
June . . .	703	6	8·5	692	8	11·5
July . . .	594	4	6·7	605	4	6·6

Experiments made in the jail showed that at depths of less than three feet the fly maggots worm their way up to the surface to pupate. If trenching is the only way to dispose of the night-soil and the fly nuisance has to be kept down, the night-soil must be buried at

a depth of at least three feet and the soil above rammed down properly. To meet this difficulty, I had a self-digesting septic tank put up on the same principle as the L.R.S. privy for the disposal of part of the night-soil at the jail. The capacity of the tank is 1,348 gallons and it digests the daily excreta of 250 convicts. The tank has been in use for the last two years and may not require any cleaning up for at least six months more. Such a disposal of the night-soil has the advantage of saving the labour of trenching, requiring less space and of substantially keeping down the fly nuisance.

## II. DINDIGUL.

Dindigul (population. 25,052) is a municipal town situated in a taluq of the same name in the Madura District. This town, which enjoys a comparatively cool climate, is half way between Trichinopoly and Madura, in the middle of an extensive and fertile plateau rising to 900 feet above the sea-level, bounded on the east by the Sirumalais and on the west by the Lower Pulneys. The rainfall is about 30 inches. The water-supply is scanty and the level of sub-soil water varies between 20 and 50 feet. There are no irrigation channels.

Dindigul has an average birth-rate of 59 per mille and a death-rate of 34 per mille per year. Fever and bowel complaints are severally responsible for 15 per cent of the total mortality in the year.

The different classes of the town population live separately in groups whose members have common habits and equal chances of infection from soil, food, water, etc. The necessary control over the communities under observation was secured through the good offices of the Sub-Collector, the Municipal Chairman, the District Superintendent of Police, the District Medical and Sanitary Officer, and the Manager of Messrs. Spencer & Co.'s Tobacco Factory; the five communities chosen being the sweepers, the police, the patients in the local hospital, Spencer's factory hands, and the boarders of the American Mission Schools. (1) The sweepers have a common occupation, practically pass the whole of their active life outdoor on soil which is heavily infected with hookworm larvæ, are dirty in their habits, and can procure only poor nourishment. (2) Though of different castes, the police constables have still much in common as regards liability to hookworm infection. They are able-bodied men, as only the fit are eligible for service after medical examination; they lead a healthy active outdoor life, go bare-footed, are equally clean in their

habits and can afford good nourishment. (3) Messrs. Spencer's factory hands form a somewhat mixed crowd, but have one common indoor occupation. (4) The school boarders constitute a disciplined group. (5) The patients in the hospital are mostly well-defined ankylostomiasis cases who have sought for indoor relief. I have added to the above a sixth group selected from the upper and middle classes of society; they go as a rule bare-footed, but their habits are clean; they live an entirely indoor life, are engaged in sedentary occupations and have a high standard of living and comfort. The members investigated in each group are small, but they throw sufficient light on the problems to be dealt with, and point out profitable lines of further enquiry.

The village of Vakampatty with a population of 1,566 was also selected to carry out our investigation.

The hookworm infection is universal, slightly higher than in the Negapatam series (Table X), and is mainly a *Necator* one (Table XI).

TABLE X.

*Percentage of hookworm infection for the Dindigul area (microscopic examination of faecal samples before treatment).*

DINDIGUL AREA.	DINDIGUL AREA CASES.			NEGAPATAM REPORT: TOWN AND VILLAGE CASES.
	Total cases examined.	Cases showing hookworm ova.	Percentage showing hookworm ova.	Percentage showing hookworm ova.
1. Town people . . . .	255	254	100·0	91·0
2. Vakampatty village . .	250	250	100·0	98·7
3. Hospital patients . . .	112	107	95·5	92·6
4. Post-mortems . . . .	12	12	100·0	100·0
5. American Mission School .	45	45	100·0	98·5



TABLE XI.

*Percentage of hookworm infection in Dindigul Town (macroscopic examination after treatment).*

Groups in Dindigul Town.	Total cases treated.	TOTAL CASES INFECTED WITH HOOKWORMS.			PERCENTAGE SHOWING HOOKWORMS.		
		A. duodenale infection, single or combined.	Necator infection, single or combined.	A. duodenale and Necator infection, single and combined.	A. duodenale infection, single or combined.	Necator infection, single or combined.	A. duodenale and Necator infection, single and combined.
1. Sweepers . . .	79	10	79	79	1.2	100.0	100.0
2. Police . . .	52	3	52	52	5.8	100.0	100.0
3. Upper and middle classes.	21	4	20	20	19.0	95.2	95.2
4. Hospital patients	112	7	107	107	6.2	95.5	95.5
5. Post-mortems . .	12	1	12	12	8.3	100.0	100.0

As it was not possible to give more than one treatment to the Dindigul cases, the figures in Table XII do not represent the total hookworm contents, and the table does not stand comparison with other intensity tables. Nevertheless the results in the table may still be compared with one another, and they show, as was to be expected, that the intensity of the infection is much greater in the sweepers than in the other classes.

TABLE XII.

*Intensity of hookworm infections in different communities of Dindigul Town (macroscopic examination for worms after treatment).*

Communities.	Total cases treated.	TOTAL HOOKWORMS REMOVED.			AVERAGE NUMBER OF HOOKWORMS PER CASE TREATED.		
		A. duodenale.	Necator.	A. duodenale and Necator.	A. duodenale.	Necator.	A. duodenale and Necator.
1. Sweepers . . .	79	30	10,001	10,031	0.4	126.6	127.0
2. Police . . .	52	4	1,099	1,103	0.1	21.1	21.2
3. Upper and middle classes.	21	4	226	230	0.1	10.8	10.9

Of the flies caught in the Dindigul Bazar, 2 per cent were found to carry hookworm ova in their intestinal contents.

The percentages for other helminthic infections are given in Table XIII.

TABLE XIII.

*Percentage of helminthic infections other than hookworm—in Dindigul area.*

Helminths.	DINDIGUL TOWN.		Negapattam report : Town percent- age.	VAKAMPATTY VILLAGE.		Negapattam report : Village percent- ages.
	Cases showing ova.	Percent- age showing ova.		Cases showing ova.	Percent- age showing ova.	
1. <i>Ascaris lumbricoides</i> . . .	154	37·3	70·4	51	20·4	45·8
2. <i>Trichiuris trichiura</i> . . .	49	11·9	44·8	19	7·6	37·0
3. <i>Strongyloides</i> . . .	12	2·9	8·3	5	2·0	7·1
4. <i>Oxyuris vermicularis</i> . . .	2	0·4	2·7	2	0·8	1·2
5. <i>Tænia solium</i> . . .	4	0·9	0·7	0	..	..
6. <i>Tænia saginata</i> . . .	2	0·4	0·2	0	..	..
♂ Total cases examined . . .	= 412			= 250		

The chief object of the investigation in Dindigul Town and the surrounding villages was to ascertain how far propaganda and free medical help can induce the population to adopt sanitary habits. Freedom from fairs, pilgrimages and the bustle of a city life marked Dindigul as one of the ideal places in Southern India where to start the campaign. It was hoped that by introducing sanitary education, taking sanitary measures and giving free medical advice and treatment, it might be possible to gain the confidence of the people and to secure their active co-operation in the work.

Sanitary education in Dindigul Town was pushed on as vigorously as possible. Lantern health lectures were given regularly two or three times a week on hookworm infection as also on other public health subjects. The lectures, advertised each time by tom-tom and by a wide circulation of notices and handbills, were given in different parts of the town so as to enable the people to attend them with the minimum of

trouble to themselves. Pamphlets were not issued as they had already been circulated by the Sanitary Commissioner. Effort was made throughout to enlist the sympathy of the educated classes. A special series of lectures for the better classes of women was given, but the attendance was invariably poor. Little encouragement was received from the school authorities. Demonstrations with the microscope failed to overcome the indifference of the people, who were unwilling to be convinced of the necessity of treatment. The municipality continued uninterested. The people do not appear to have adopted more sanitary habits, as may be expected from an appreciation of the propaganda work. The general sanitary condition of the town has not visibly improved. When work was stopped on May 24, 1919, the soil pollution in the town was much the same as at the start.

The relieving feature of the campaign was the popularity of the lantern lectures in the villages, where they were largely attended. The villagers seemed to have realised that the restrictions of sanitation were an evil preferable to the suffering from diseases; and, in the cholera epidemic of January 1918, they did try to observe the few simple rules I laid down for them. They understood, too, that sanitation must be observed by the many in order to obtain a proportionate benefit from it, and this served to bring them for a time in closer union. Thus matters seemed to have taken a favourable turn, when in November 1918 influenza broke out. They unanimously assumed that this was God's visitation and that no medicine was needed since those who were to die had already been marked out. No amount of persuasion could shake the villager's apathy.

One serious obstacle to the progress of sanitation in villages is poverty. With the limited resources in their hands, the villagers cannot afford to put up latrines for the prevention of soil pollution, nor can they adopt any measures for the conservation of the water-supply or the improvement of the drainage. A further drawback is the want of a free space where to build privies for the family use. Most of the houses are one-roomed huts without any surrounding open space. It is thus impossible to suggest to a house-holder, however willing he may be, to build a privy, for he has no place complying with the sanitary regulations. The latrines have necessarily to be erected outside the villages on public land. In Vakampatty, which normally requires eight such, two have been constructed to bring home to the inhabitants the benefits of sanitation. Though these two were in regular use, there was no demand for

more. The type selected for trial was the latest type design 112 c. of the Madras Sanitary Board (dry earth type).

Protection of the water-supply from the polluted soil is another necessity in these parts. The wells are almost always without any parapet walls or any brick-lining. The water is drawn up by standing over a grating on the well and pulling up the bucket. In addition to the percolation of water through the infected soil, much of the soil itself is washed directly into the well from the feet of the persons and is taken away in the water meant for drinking purposes. The Local Fund Board have, at my request, provided with pumps the two wells at the village of Vakampatty and improved the surrounding drainage.

In Dindigul Town, two trial latrines have been built in the quarters inhabited by Messrs. Spencer's factory hands who realised the necessity for the same. Both the designs erected there are giving satisfaction.

Free medical advice and treatment was no allurements for the town-people, and few were those who applied to us for help. Medical assistance to be appreciated by the villagers had to be taken to their very doors. A free dispensary opened at Vakampatty soon attracted patients from the neighbouring villages within a radius of ten miles. But they came more for the alleviation of complaints which they could see or feel than for deliverance from an infection they refuse to believe in. Their notion of normal health includes hookworm infection.

I found ground itch to be entirely absent from these districts: 32 per cent of the patients had skin diseases, mostly scabies; 18 per cent had digestive troubles; 7 per cent had irregular pains in the joints. Pure ankylostomiasis uncomplicated by tuberculosis, malaria, kala-azar, etc. was found in 6.3 per cent of the dispensary patients. 6 per cent had eye troubles. I had occasion to notice that ankylostomiasis, digestive troubles, general debility and pseudo-rheumatism cases were much improved by the hookworm treatments. The two great drawbacks to the treatment from the villagers's point of view were: (1) the necessity of laying aside at least half a day; and (2) the necessity of taking the drug very often as the re-infection from the polluted soil is so persistent.

For the prosecution of the village campaign I was backed up by the best of assistance, as the revenue authorities, the village officers and the parish priest were always eager to further my plans. Never did I meet with systematic opposition, but, throughout the campaign, the inertia of the people consequent upon their ignorance proved a decided hindrance. Lack of confidence in the 'foreign' doctor may also partially account

for the apparent apathy. I remember one of the lectures at Dindigul taking the wrong turn, upon a much travelled old butler remarking that 'he had never seen such a worm in any part of the world.'

#### CONCLUSIONS.

(1) This report confirms the results of the inquiry carried out among the Tanjil coolies collected from the southern half of this Presidency at the Negapatam Emigration Dépôt. The percentage of the hookworm infection is nearly 100 per cent in the four districts of Tanjore, Trichinopoly, Madura, and Ramnad. Of the two species of hookworm harboured—*Necator americanus* and *Ankylostomum duodenale*—the percentage of the former is nearly 100, while that of the latter varies widely, being 80 per cent in Tanjore and 10 per cent in Madura districts.

(2) The hookworm infection, though universal, varies in intensity in the various districts, as also in the various communities. The highest infection met with so far is in Tanjore District with an average of 91 *Necator* and 10 *A. duodenale* per person.

(3) Freedom from hookworm infection seems to diminish the susceptibility to bowel complaints.

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THE CORRELATION BETWEEN THE CHEMICAL COMPOSITION OF ANTHELMINTICS AND THEIR THERAPEUTIC VALUES IN CONNECTION WITH THE HOOK-WORM INQUIRY IN THE MADRAS PRESIDENCY.

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INTRODUCTION.

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HISTORY.

COMPOSITION AND PROPERTIES.

ABSORPTION AND ELIMINATION.

THE RATIONALE OF THYMOL TREATMENT WITH REFERENCE  
TO ITS ABSORPTION AND ELIMINATION.

THE RATIONALE OF THYMOL TREATMENT WITH REFERENCE  
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THE EFFICIENCY OF THYMOL TREATMENT.

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INTRODUCTION.

IN this and future papers the term *anthelmintic* will apply in general to any medicine against worms, whether it destroys the intestinal

parasites, or simply promotes their expulsion, or does both. The term *vermicide* will be restricted to drugs which kill the worms; and the term *vermifuge* will be used to denote a remedy which expels worms from the animal body, without necessarily killing them.

Anthelmintics are substances the value of which has been determined empirically, and which are believed to possess the property of reaching the intestinal tract without being absorbed to any great extent. Unfortunately, their toxicity is by no means specific, and their action on the worms is quite generally associated with a poisonous effect on the host. All that can be done in these conditions is to try and decide for every particular kind of infection which anthelmintic shows the maximum toxicity towards the parasites and the minimum toxicity towards the host. This has always been the guiding principle underlying the therapeutic of helminth infections.

On the assumption that anthelmintics are very little soluble in the intestinal secretions and can, with due precautions, be prevented from being absorbed in the system in toxic quantities, they are administered in large doses, and efficient steps are taken to remove the unabsorbed portion rapidly from the system. This last result largely depends on the judicious use of purgatives which eventually may also act as vermifuges. The dosage of the anthelmintic and the time at which this after-purge is to be given are conditioned by the habits of the parasites and their location in the intestine. Thus the position of the hookworms high up in the intestinal tract justifies the use of very large doses of anthelmintics, as the possibilities of absorption are really small owing to the short length of intestine the drug has to travel through to come in contact with the parasites and destroy them. Equally large doses are not to be recommended in the case of round-worm infection, because of the long way the drug has to go before reaching the worms; and medication by the mouth is not to be thought of in the case of whip-worms and thread-worms which are to be found in the cæcum, or near the rectum, respectively.

In the treatment of hookworm infection the anthelmintic is administered in doses lethal to the worms in such strong concentrations as to saturate the intestinal contents and, as much as possible, to ensure contact with the parasites. This direct action of the drug is further assured by keeping the intestinal tract empty and adopting such other means as will set the worms free from the mucus which surrounds them.



The present-day rationale of hookworm treatment is, therefore, the following:—(1) to administer an alkaline mixture so as to remove the intestinal mucus which surrounds the worms; (2) to starve the patient or keep him on a low diet so as to have the intestine free from food debris and secretions which would retard the action of the anthelmintic; (3) to wash out the intestine and expose the worms by means of a purge; (4) to administer the anthelmintic in such a dose as is deemed necessary for the destruction of the worms, and to repeat this at definite intervals in order to keep the drug at the same degree of concentration; (5) to starve the patient and prevent him from partaking of any substance which will dissolve the anthelmintic and thereby increase its absorption; (6) to expel the unabsorbed portion of the toxic drug and eventually the worms by means of a purge; (7) to prohibit the use of solvents till the complete removal of the anthelmintic.

The long time required for such a treatment and the precautions to be observed in employing the anthelmintic necessitate hospital conditions and the attendance of the medical man. This is more than sufficient to render the treatment unpopular with the masses, and it is to be deplored all the more as hookworm infection is universal in this Presidency.

The procedure of treatment just detailed was started with a view to meet those infections detected in the old days by the smear method—infections which were undoubtedly very heavy; but highly infected cases are not the only ones to call for intervention. As pointed out by Clayton Lane (43), we must admit with the Rockefeller Commission that even very mild infections constitute a grave handicap to the unfortunate harbourers and are of clinical as well as of sanitary importance. The procedure of treatment was moreover suited to remove the hardier hookworm, *Ankylostoma*. Whether the mild infections of less than 100 or 50 worms, mostly *Necator*, noted now-a-days justify the precautions taken to prepare the intestinal tract and the removal of the worms, becomes, therefore, a debatable point.

Our object is to discuss this mode of treatment for each of the anthelmintics used in hookworm infection.

The literature on the subject shows that authors are far from being at one on the respective value of the drugs. This want of agreement chiefly bears on three points, which are all dependent upon the composition of the substance: the dosage, the destructive action on the parasites, and the toxicity towards the host. Some of the drugs are chemical

compounds with well-defined composition and properties, others are mixtures in which the anthelmintic agent may be found in variable quantity: some keep well almost indefinitely, others deteriorate with age. It is then evident that results may be different not only with different substances but also with the same. Again, many of the results appear as mere figures without reference to the age, sex, nationality, and 'health' of the infected individuals, or to the nature of the infection. These, however, are factors which ought to be carefully balanced when results are to be compared. For instance, patients belonging to different ethnic stocks may harbour different entozoa with the inevitable consequence that an anthelmintic may prove very efficient with a particular group and most disappointing with others; moreover, limit of tolerance to the drug will be found to vary in that particular group with the age, sex, and 'health' of the person under treatment.

Nor is there any agreement as to the way of interpreting the efficiency of anthelmintics. Not less than three methods seem to have been adopted: (1) method based on the percentage of hookworms removed with one test treatment; (2) that based on the percentage of cases cured with one test treatment; (3) that based on the average number of treatments required to cure a group of infected persons. The term 'cure' has not, to our knowledge, been defined; but from the statistics, it appears that the absence of hookworm ova from the faeces ten days or more after a treatment has generally been taken as an indication of 'cure.'

Further, the liberties that have been taken in the past with the dosage and after-treatment led us to inquire into the justification of the same, and then to attempt to balance the toxic action of each drug with its anthelmintic value, in such a way that the mode of treatment should find favour with the masses and meet the conditions of a light and wide infection in an apparently healthy community.

Taking, as far as possible, the above facts into consideration we have adopted the following procedure as the one most likely to enable us to reach the end we have in view:—(1) to examine the chemical nature of the anthelmintic; (2) to determine its toxicity towards the host; the rate and amount of absorption, if any; its neutralisation in the system; its elimination by the kidneys and in the faeces; (3) to determine its action on the hookworms whether vermicide or vermifugal, or both.

We need not emphasize Clayton Lane's<sup>(43)</sup> conviction that 'in undertaking the freeing of India from hookworm infection one is faced by the prospect of treating at least 250,000,000 persons and that the

country is a poor one ; that the unit of cost will prove the most important consideration with those on whom will fall the decision as to the drug to be used and the method of its administration.'

As ours is a prolonged inquiry, we think we are justified in publishing occasionally such results as, in our opinion, may help in the extirpation of the disease, even before some of the points at issue are satisfactorily cleared.

## I. THYMOL.

### HISTORY.

(A) In 1879, Bozzolo<sup>(3)</sup> successfully treated with thymol a case of ankylostomiasis which had proved refractory to the extract of male fern, and started administering the drug freely.

Parona<sup>(4)</sup>, in 1881, not only confirmed the value of thymol as an anthelmintic in ankylostomiasis, but also stated that the female worms were more easily expelled than the males.

In 1887, Sandwith, of Cairo, began treating his cases successfully with thymol, having previously had nothing but disappointment from the male fern and santonin then in vogue<sup>(7)</sup>.

From that time onwards the efficacy of the drug was repeatedly asserted, and thymol became the universal drug for ankylostomiasis for many years. The Porto Rico Commission (1899-1910) recommended its use in doses per age group. They also sometimes treated pregnant women with the drug to save life and on several occasions nursing mothers were given thymol without bad effects to either mother or child<sup>(32)</sup>.

Stiles (1903)<sup>(9)</sup> recommended the use of thymol in the American hookworm infection ; and Allen J. Smith (1904)<sup>(12)</sup> declared that 'at the time no more efficient remedy than thymol is known.'

Even after the introduction of beta-naphthol, eucalyptol, and chenopodium oil, thymol is for many the drug *par excellence*. Colbert (1908)<sup>(17)</sup>, Thomas (1909)<sup>(23)</sup>, Fussell<sup>(21)</sup>, Lindeman (1910)<sup>(22)</sup>, Schultz (1911)<sup>(26)</sup>, Wyler<sup>(30)</sup>, Ashford (1913)<sup>(27)</sup>, Blin<sup>(31)</sup>, Macallan<sup>(34)</sup>, Schuffner and Vervoort (1914)<sup>(29)</sup>, Deaderick<sup>(32)</sup>, Clayton Lane (1915)<sup>(33)</sup> admit that thymol is, when properly employed, a very certain means of causing the expulsion of parasites and that its use is unattended with danger, if it be administered with the observance of certain simple and necessary precautions.

At present thymol treatment in infected areas is almost general.

(B) Bozzolo gave at first 2 to 10 grammes of powdered thymol a day; he later gave 2 grammes every 2 hours until 12 grammes were taken.

The routine treatment in the Egyptian Hospitals (1898)<sup>(35)</sup> was to place the patient on a milk diet for a whole day on the evening of which he was dosed with one gramme of thymol. Early the next morning the thymol dose was repeated and followed by 30 grammes of Epsom salts. Sandwith considered that 4 grammes in 24 hours are as efficacious as 6 grammes and the former dose is certainly less dangerous.

Leichtenstern (1898)<sup>(13)</sup> gave as much as 15 grammes of thymol.

Stiles (1903)<sup>(10)</sup> advocated a milk and soup diet for three days previous to the day of treatment, when 2 grammes of thymol were given at 8 A.M.; 2 grammes at 10 A.M.; and castor oil or magnesia at 12 noon. Bentley<sup>(11)</sup>, in Assam (1904), also gave it in the same dosage.

Calmette and Breton<sup>(13)</sup> advise its administration in two-hourly doses of 2 grammes each until 6 to 8 grammes have been given.

Clayton Lane (1909)<sup>(19)</sup> recommended 3 doses of 30 grains each at intervals of one hour. Brumpt (1910)<sup>(20)</sup> advocates the use of  $\frac{1}{2}$  to  $1\frac{1}{2}$  grammes of thymol in capsules fixing 10 grammes as the maximal dose.

Fergusson<sup>(6)</sup>, in British Guiana, gave 10 grain doses every night for six days in the week until 100 doses were taken. Saundby (1912)<sup>(28)</sup> gave to one of his patients as much as 120 grains in two doses of 60 grains each. Nicol (1912)<sup>(26)</sup> gave 3 doses of 30 grains each every 2 hours followed by a purge at the end of 6 hours.

Ashford (1913) treated nearly 2 million cases with 60 grains of thymol in 2 doses at two-hourly intervals. Wyler gave thymol in 3 doses of 30 grains each for 3 hours and gave the after-purge 8 hours after. Schuffner and Vervoort (1913) gave one gramme every hour till 5 grammes were taken and followed it by castor oil. The Pacific Mail Steamship Company (1916)<sup>(30)</sup> gave 80 grains in the morning in four-hourly doses of 20 grains each; one and a half ounce of castor oil the night preceding with one ounce of Epsom salts two hours before and one hour after administration. Some of their patients took as much as 1,720 grains of thymol in a period of 26 days.

Snedgrass (1916)<sup>(40)</sup>, in Ceylon, following the Rockefeller Commission, gives 60 grains in two doses of 30 grains each. Darling, Barber and Hacker (1918)<sup>(46)</sup> have as an experimental measure given 180 grains in 3 one-hourly doses of 60 grains each.

The dosage at the General Hospital, Madras, since 1910, has been 90 grains in two 45-grain doses given at two hours' interval. Clayton Lane (1916)<sup>(38)</sup>, in his Darjeeling Investigation, gave 60 grains in 3 portions at intervals of one hour, a practice which we have been following to this day as a 'standard treatment'.

(C) Bozzolo gave powdered thymol and followed it by a weak alcoholic beverage, so as to have a weak solution of the drug in the alimentary tract. Thirty years later he is still of opinion that his patients were not poisoned and thinks the thymol was probably more effective when administered in this way. Fergusson administered thymol in cachet or tablet form. Lindeman (1911) pointed to the risk attending such a procedure: namely, the drug failing to reach the parasite before it is propelled by the purgative. The Rockefeller Commission (1914) emphasised the necessity of giving the drug freshly powdered and in a particulate form with sugar of milk. More recently (1917) they have recommended its use with soda-bicarb powder. We have been using thymol freshly powdered with cane-sugar.

Sandwith, on the other hand, gave alcoholics, not as a solvent of thymol so as to increase the action of the drug, but because 'warned by the death of at least one of my patients immediately after digesting the thymol, I have always administered 25 grammes of brandy with each two grammes of thymol (at 8 A.M. and again at 10 A.M.) with the happiest results.' Stiles from experiments on dogs (1903) concluded against the administration of alcoholics during treatment.

Ashford (1913) mentions that about 2 million doses of thymol were distributed in Porto Rico without a fatality, the people taking the drug home with them and undoubtedly drinking rum afterwards.

Thornhill (1895) is quoted by Manson (1915)<sup>(44)</sup> as having related an instance in which a fatal result was brought about by the ingestion of arrack just after administering the dose of thymol.

All recent writers on the subject are unanimous in cautioning against the use of alcohol or other solvents of thymol during treatment with this drug.

(D) Whatever the dosage or mode of administration fatal issues with thymol seem to have been rare. Sandwith (1894), quoted by Stiles, records that out of 8 fatal cases treated, two died he thinks in consequence of the thymol, 11 and 48 hours respectively after taking the dose. They were in a miserable state of exhaustion and debility. He does not think that thymol accelerated the death of any one of the remaining cases.

Ashford (1913) with his vast experience personally believed that many of the cases of so-called poisoning which have been chronicled as having been caused by thymol are either due to unwarranted overdose or to excessive purging in a weak and anæmic patient, thus precipitating an exhausting and fatal diarrhœa.

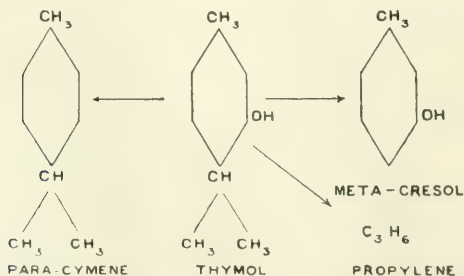
These are the only two deaths mentioned as being due to thymol. No other was found recorded in the literature we could get at.

#### COMPOSITION AND PROPERTIES.

Thymol, melting at  $50^{\circ}$  and boiling at  $232^{\circ}$ , crystallises in large transparent plates, and has a peppery taste, with a mild pleasant odour, like that of thyme. It is sparingly soluble in water—1,100 parts of water at  $16^{\circ}$  and 900 parts of boiling water,— but very soluble in alcohol, glycerine, ether, petroleum, chloroform, carbon disulphide, turpentine, oils and fats, soaps and alkaline solutions, and acetic acid.

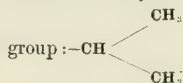
It exists, with cymene and thymene, in oil of thyme from *Thymus vulgaris* and *Th. serpyllum*, and in the oils of *Carum copticum*, an East Indian plant, *Monarda punctata*, or Oswego tea, a native of North America, and *Mentha viridis*. It may either be extracted from these oils or artificially prepared from cuminaldehyde, the chief constituent of cumin oil—from *Cuminum cyminum*, a plant cultivated in most provinces of India. Thymol is now an article of Indian manufacture.

Heated with phosphoric anhydride, thymol yields propylene and meta-cresol; while, when treated with phosphorus pentasulphide, it yields para-cymene. These two reactions indicate that the groups contained in thymol bear to each other the relations shown by the formulæ given below :

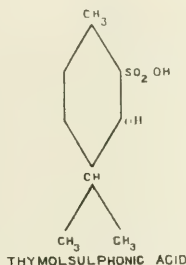
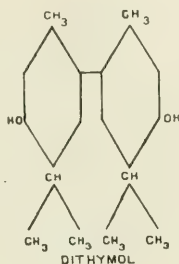
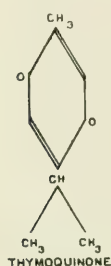




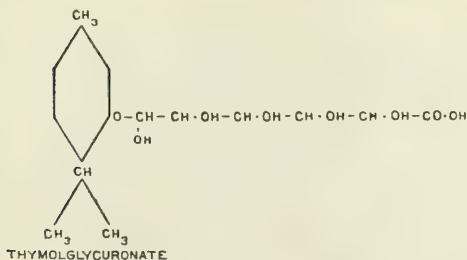
The synthetic methods of preparation and the various modes of formation of thymol clearly prove that the radicle  $C_3H_7$  is the isopropyl



Chromic acid oxidises thymol to thymoquinone; while, ferric chloride and oxidising ferments (16) convert it into dithymol. Thymol dissolves in cold sulphuric acid forming a yellow solution which, on heating, assumes a red-orange colouration due to the formation of thymol-sulphonic acid.

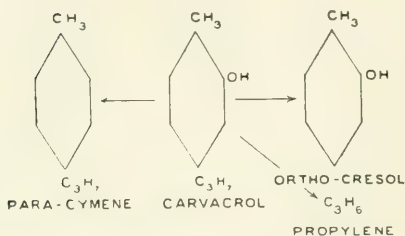


Thymol condenses with glycuronic acid without elimination of water.

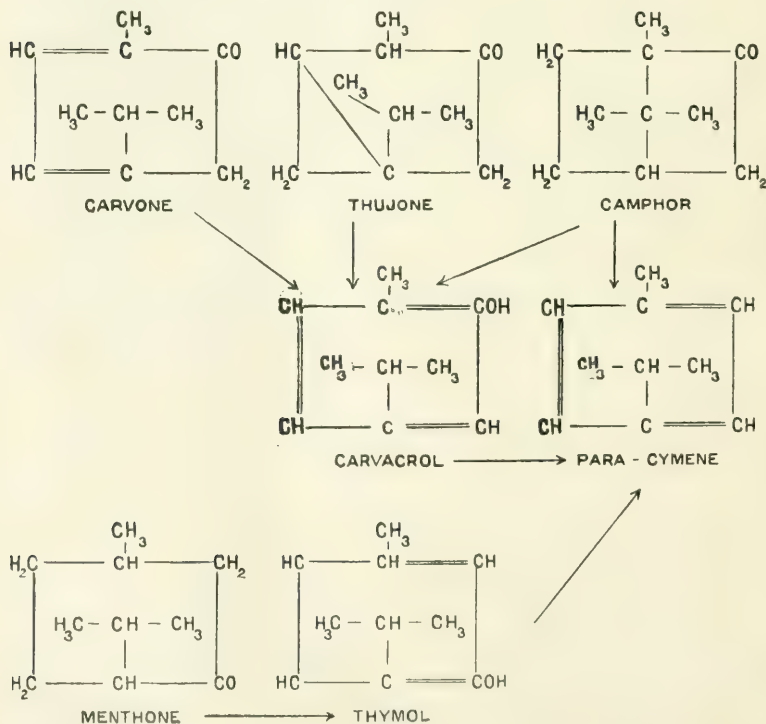


Thymol is one of the two theoretically possible hydroxyl derivatives of para-cymene. The other one, carvacrol, has the hydroxyl in the ortho position relatively to methyl.

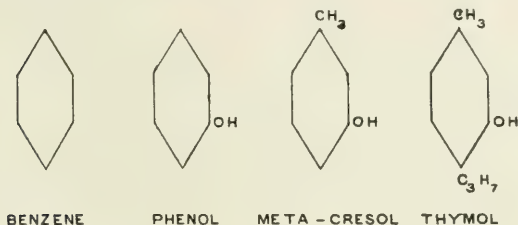




Through para-cymene and carvacrol thymol is closely related to many of the terpenes, or hydrocarbons of the turpentine class, and their oxygenated derivatives, the camphors.



Thymol may be looked upon as one of the higher homologues of benzene, phenol, or metacresol.



As a matter of fact its physiological properties are but variations of the properties exhibited by those three substances. It is a powerful antiseptic, acting as a caustic irritant of the mucous membrane and causing excitant effects in the alimentary canal. Its toxic effects are similar to those of phenols, but of a somewhat milder grade. In poisoning from its use, locally there is a burning sensation in the stomach and slightly caustic effects on the mucous membrane of the intestines, producing intestinal irritation and evacuation of fæces, nausea and vomiting. Tremor and convulsions are induced, though rarely, and are less intense than those induced by phenol.

Thymol is a drug of uniform quality, and one that keeps well almost indefinitely.

#### ABSORPTION AND ELIMINATION.

*Previous investigations.*—Experimenting with dogs, Seidell<sup>(36)</sup> has shown that thymol is absorbed in the system and reappears in the urine as a glycuronate, just as other phenolic derivatives combine with glycuronic acid in the metabolism. Complete elimination of the glycuronate takes place within 24 hours from the time of ingestion, and the amount of absorbed thymol thus recovered is found to vary from 31 to 46 per cent. As only insignificant quantities of the administered thymol are excreted with the fæces, from one-half to two-thirds is apparently destroyed or fixed in the body. A similar fate has been suggested for compounds of related type, such as the simpler phenols, but no satisfactory explanation has, as yet, been found for this apparent disappearance of administered phenols.

*Procedure adopted.*—Healthy male convicts from the Trichinopoly Central Jail were selected for the purpose of our investigation. They were given a light diet at 5 P.M. followed by a magnesium sulphate purge at 8 P.M. The next morning they were asked to urinate and were then treated with freshly powdered thymol. Epsom salt was given two hours after administration of the last dose. The urine and faeces discharged after treatment were separately collected for analysis.

Note was taken of the colour, volume, density, and acidity of the urine, which was clinically examined for albumin, phenols, and indican. A measured quantity was then subjected to double distillation, acid and neutral, and the amount of thymol recovered was calculated for every 24 hours.

A watery faecal extract was prepared by collecting the stools in three litres of water holding 15 grains of copper sulphate in solution, and extracting for a day with frequent shaking. This extract was examined for thymol.

The species, number, and the condition of the hookworms expelled after the test treatment were noted, and the patient's total hookworm content was determined by repeating the thymol treatment until cure followed.

Variations in the dosage of the anthelmintic and in the method of treatment were tried.

*Experiments.*—1. The different samples of thymol we have been using were all found to be chemically pure.

2. Aqueous solutions containing known quantities of thymol were titrated according to the bromine method proposed by Seidell, as follows:—About 2 c.c. of carbon tetrachloride were added to the thymol solution, and bromine vapour poured in with active shaking, until a permanent red colour of the carbon tetrachloride layer was obtained. The flask was then allowed to stand in a dark place for about half an hour. 6 c.c. of carbon disulphide and 5 c.c. of 20 per cent potassium iodide solution were added and the liberated iodine titrated with standard decinormal thiosulphate solution. 5 c.c. of a 2 per cent potassium iodate solution were then added and the liberated iodine corresponding to the free hydrobromic acid was titrated with the same standard thiosulphate. The difference between the first and the second readings of thiosulphate solution corresponds to the hydrobromic acid formed in the bromination of thymol. The calculation is made on the basis of two molecules of hydrobromic acid for one molecule of thymol;

1 c.c. decinormal thiosulphate is, therefore, equal to 0.0075 gramme thymol.

Our results showed that this method is a perfectly reliable one for the estimation of thymol.

3. Definite and varying amounts of thymol, determined from the quantities usually recoverable from 100 c.c. of urine after thymolization, were suspended in water and subjected to Seidell's double distillation process as follows:—Steam was passed through a distillation flask containing the thymol with 180 c.c. of distilled water and 20 c.c. of concentrated sulphuric acid. The distillate was received in a second distillation flask containing 5 grammes of copper sulphate dissolved in 100 c.c. of water to which was added 2 grammes of magnesium oxide. An efficient condenser followed this second flask and 100 c.c. of distillate was collected in a glass-stoppered receiver in which the titration of the thymol was made directly by the bromine-hydrobromic acid method.

It was thus found that during distillation a destruction of some of the thymol occurred, and that this destruction was roughly proportional to the amount of substance present. This would, therefore, constitute an error which we have called the 'minus error.'

TABLE I.

*Percentage of thymol recovered, and 'minus error' due to partial destruction of thymol during distillation.*

Experi- ment No.	Thymol taken.	Thymol recovered.	Thymol recovered per cent.	Thymol lost or 'minus error.'
	mg.	mg.	%	mg.
1	49.0	46.5	94.9	2.5
2	109.5	102.7	93.8	6.8
3	144.0	130.5	90.7	13.5
4	213.3	198.0	92.9	15.3
5	383.1	347.9	90.8	35.2
Average..	..	..	91.5	14.66

4. It is well known that normal urine, when subjected to distillation, yields varying amounts of substances which in the titration react just as

thymol. This would, therefore, constitute a 'plus error.' A number of blank distillations were accordingly carried out, with a view to determine correction factors. Bromine reacting substances have been considered throughout as equivalent to thymol, the object of our investigation, and calculations are based on the assumption that 1 c.c. of decinormal thiosulphate solution is equal to 0.0075 gramme of the substance. The results are shown in Table 2.

TABLE II.

*Amount of substances reacting like thymol in the titration, and 'plus error' due to their presence in normal urine.*

Case No.	Total No. of experiments in each case.	BROMINE REACTING SUBSTANCES IN NORMAL URINE.				
		Average volume of urine in 24 hours.	Average for 24 hours in mg.	Maximum passed by the case.	Minimum passed by the case.	'Plus error' for 100 c.c. of urine.
		c.c.	mg.	mg.	mg.	mg.
1	3	1,561	135.5	154	123	8.6
2	3	2,325	275.6	312	221	11.8
3	4	1,887	172.7	277	108	9.1
4	4	1,661	229.7	311	167	13.8
5	5	1,153	370.2	470	325	32.1
6	5	1,635	163.8	211	128	10.0
7	4	1,916	279.0	341	225	14.5
8	4	1,741	249.5	319	146	14.3
9	5	2,034	176.6	221	151	8.6
10	5	2,074	274.5	299	252	13.2
11	1	2,414	407.0	—	—	16.8
12	1	662	173.0	—	—	26.1
13	1	2,406	324.0	—	—	13.4
Average	..	..	..	..	..	14.61

Thus the amount of bromine reacting substances present in the 24 hours' urine of presumably normal adults is found to vary within wide limits not only for different subjects but also for the same individual from day to day. It is evident from this that an attempt to determine correction factors would prove futile. If, however, we calculate the

'plus error' for 100 c.c. of urine—this being the quantity to be distilled for the estimation of thymol in thymolated urine,—we see that this 'plus error' balances to a certain extent the 'minus error' due to the loss of thymol during distillation. The uncorrected results, as reported later in the tables, may, therefore, be taken as fairly representing the maximum amounts of thymol excreted in the urine in combination with glycuronic acid.

The apparatus used was the same as the one employed for the determination of the 'minus error.' The acid distillation flask contained 100 c.c. of urine with 80 c.c. of water and 20 c.c. of sulphuric acid.

5. The next point of interest was to see the effect a cathartic might possibly have on the elimination of the bromine reacting substances. Eight convicts so far untreated for hookworm infection were accordingly selected and given a purge consisting of six drachms of magnesium sulphate. The 24 hours' urine was collected and submitted to the process of distillation already described.

TABLE III.

*Volume of urine passed in 24 hours, and amount of bromine reacting substances excreted in the same time after a magnesium sulphate purge.*

*(One experiment in each case, vide Table II.)*

Case No.	Volume of urine for 24 hours.	Specific gravity.	BROMINE REACTING SUBSTANCES PASSED IN 24 HOURS.	
			Total quantity in mg.	'Plus error' for 100 c.c. of urine.
	c.c.		mg.	mg.
1	1,641	—	233	<b>14·2</b>
2	2,240	—	302	<b>13·4</b>
3	1,528	1,010	240	<b>15·0</b>
4	1,206	1,012	235	<b>19·4</b>
5	1,054	1,020	347	<b>32·8</b>
6	1,760	1,010	170	<b>9·6</b>
7	1,702	1,012	306	<b>17·9</b>
8	1,270	1,011	95	<b>7·4</b>

TABLE IV.

*'Plus error' variations in 100 c.c. urine for Table II cases.*

Case No.	Possible variation from day to day in mg.		Before a purge.	After a purge.
	mg.	mg.	mg.	mg.
1	8.9	8.2	<b>8.6</b>	<b>14.2</b>
2	17.9	9.4	<b>11.8</b>	<b>13.4</b>
3	5.9	14.9	<b>9.1</b>	<b>15.0</b>
4	10.4	25.4	<b>13.8</b>	<b>19.4</b>
5	29.2	37.5	<b>32.1</b>	<b>32.8</b>
6	6.7	11.2	<b>10.0</b>	<b>9.6</b>
7	11.9	18.7	<b>14.5</b>	<b>17.9</b>
8	7.4	17.8	<b>14.3</b>	<b>7.4</b>

Tables III and IV show that the administration of a purgative does not appreciably affect the normal elimination of substances reacting with bromine.

6. Watery faecal extracts were also submitted to the double distillation process and were found to contain bromine reacting products: the average in normal 24 hours' faeces being 579 mg.

7. By allowing definite amounts of dissolved thymol to remain in intimate contact with a mass of faeces for 24 hours we were able to ascertain that no appreciable reduction in the quantity of thymol obtainable did occur. Any thymol existing free in the intestinal tract ought, therefore, to be recovered by distillation. And, as a matter of fact, we were able to recover 95 per cent of the drug from such a small quantity as half grain.

Let it be noted here that thymol begins to distil over as soon as the contents of the second distillation flask have reached their boiling point and that its presence, even in small quantities, is invariably shown by the opalescence of the distillate. With larger quantities a certain amount of beading may be noticed in the condenser and globules are to be seen floating on the surface of the distillate. The beading, the opalescence of the distillate and the formation of globules were never found to occur with the bromine reacting substances in the absence of thymol, however large their amount.



THE RATIONALE OF THYMOL TREATMENT WITH REFERENCE  
TO ITS ABSORPTION AND ELIMINATION.

1. *Time taken for complete thymol elimination.*—(a) 60 grains of thymol in powder, with sugar finely divided, were administered in three portions to ten cases. The urine was collected for every period of 24 hours from the time of the administration of the first dose of thymol during six consecutive days. Thymol was only detected in the first 24 hours' sample.

(b) Four cases were treated as above and their urine was collected for a period of 48 hours after the administration of the first dose of thymol, at regular intervals. The portion passed within the first five hours constituted the first sample, that within the next six hours the second, and that passed during the night, 13 hours, the third. With a view to determine the end point of elimination the samples were collected on the second day at regular intervals of four hours, and the night urine formed an additional sample. It was thus found that all the available thymol is eliminated during the 24 hours which follow the treatment. (Table V.)

TABLE V.

*Amount of thymol excreted at different periods (by hours) after treatment with 60 grains of thymol.*

Number of cases examined.	Time of urine collection after thymol treatment.	Average volume of urine for the period.	Average of thymol recovered in the period.	Percentage of thymol recovered to total thymol given.
	Hours.	c.c.	mg.	
4	0—5	331	313.5	<b>8.06</b>
4	5—11	205	317.75	<b>8.17</b>
4	11—24	695	266.75	<b>6.86</b>
3	24—28	186	0.0	<b>0.0</b>
3	28—32	303	0.0	<b>0.0</b>
3	32—36	98	0.0	<b>0.0</b>
3	36—48	872	0.0	<b>0.0</b>

(c) No thymol could be recovered from the faeces.

2. *Effect of the dosage on the elimination of thymol.*—Twelve series of convicts were treated with 10, 20, 30, 40, 50, and 60 grains of

thymol. Six batches received the drug in three portions, while the other six received it in one portion only.

(a) The 24 hours' urine was collected in each case and the amount of thymol eliminated during that time was determined. The results are shown in Tables VI and VII.

TABLE VI.

*Total and average amounts of thymol recovered from the urine. (Thymol given in three portions.)*

Experiment No.	Total number of cases examined.	Thymol given in three portions.	Average volume of urine in 24 hours after treatment.	Average specific gravity.	Average of thymol recovered.	Percentage of thymol recovered.	THYMOL RECOVERED.	
							Maximum.	Minimum.
		Grains.	c.c.		Grains.		Grains.	Grains.
1	12	10	1,151	1,013	4.4	43.7	6.97	2.08
2	15	20	838	1,015	5.3	25.1	7.28	3.84
3	12	30	828	1,016	7.8	26.1	10.83	5.47
4	12	40	720	1,015	7.8	19.5	12.73	4.53
5	12	50	695	1,017	11.7	23.3	19.47	7.19
6	13	60	1,028	1,014	14.1	23.5	19.12	8.48

TABLE VII.

*Total and average amount of thymol recovered from the urine. (Thymol given in one portion.)*

Experiment No.	Total number of cases examined.	Thymol given in one portion.	Average volume of urine in 24 hours after treatment.	Average specific gravity.	Average of thymol recovered.	Percentage of thymol recovered.	THYMOL RECOVERED.	
							Maximum.	Minimum.
		Grains.	c.c.		Grains.		Grains.	Grains.
1	12	10	838	1,016	4.2	42.7	5.57	2.11
2	12	20	937	1,014	6.0	30.0	8.59	3.50
3	12	30	889	1,014	7.1	23.7	12.48	4.30
4	12	40	904	1,016	9.2	22.9	13.68	5.52
5	12	50	816	1,017	11.1	22.1	17.53	5.58
6	18	60	1,241	1,014	12.7	21.2	17.17	9.32

As seen from these tables :—

(i) The amount of thymol absorbed in the system and eliminated by the kidneys is proportional to the amount of the drug ingested.

(ii) The percentage of thymol recovered is higher for the smaller than for the larger doses, and is fairly constant for doses of 30 grains and above.

(iii) There is a wide variation in the amount recovered from the same dose.

(iv) The amount of thymol eliminated depends on the quantity administered and not on the mode of administration.

(v) There is no relation between the amount of thymol recovered and the volume or the density of the urine.

(b) In 16 cases the faeces excreted during the 24 hours following the treatment were collected and examined for thymol. No trace of the drug could ever be detected in the faecal extracts.

TABLE VIII.

*Amount of 'bromine reacting substances' other than thymol, recovered from 24 hours' faeces at the time of thymol treatment (excepting experiment 16, thymol 60 grs. was given in one portion).*

Experiment No.	Thymol dose.	Time when purge was given after the last thymol dose.	Thymol recovered in the urine.	'Bromine reacting substances' recovered from 24 hours' faeces.
1	20 grains .	2 hours . . .	4.94 grains .	382.5 mg.
2	30 " .	3 " . . .	7.51 " .	135.0 "
3	30 " .	2 " . . .	4.97 " .	922.5 "
4	30 " .	2 " . . .	6.09 " .	652.5 "
5	50 " .	2 " . . .	7.87 " .	157.5 "
6	50 " .	2 " . . .	10.24 " .	202.5 "
7	60 " .	After-purge and thymol together.	17.17 " .	Not calculated.
8	60 " .	Ditto .	13.87 " .	Ditto.
9	60 " .	$\frac{1}{2}$ hour . . .	12.26 " .	337.0 mg.
10	60 " .	$\frac{1}{2}$ " . . .	11.40 " .	810.0 "
11	60 " .	1 " . . .	14.25 " .	Not calculated.
12	60 " .	1 " . . .	10.37 " .	Ditto.
13	60 " .	1 $\frac{1}{2}$ hours . .	Not calculated .	Ditto.
14	60 " .	1 $\frac{1}{2}$ " . . .	Ditto .	Ditto.
15	60 " .	2 " . . .	10.98 grains .	832.5 mg.
16	60 " .	3 " . . .	10.15 " .	765.0 "

Table VIII shows the amounts of bromine reacting substances present in the fæces after treatment with thymol. Those amounts vary greatly with different individuals and do not appear to be in any way influenced by the quantity of the drug ingested—a fact corroborated by the figures obtained in the case of untreated subjects, *viz.*, 810, 765, 261, 480 mg.

3. *Effect of the after-purge on the elimination of thymol.*—(a) All the cases shown in Table VI were given the dose of thymol in three equal portions at hourly intervals and a purge of saline was administered two hours after the ingestion of the last portion. The cases figured in Table VII were purged three hours after the administration of the drug in a single portion. We have already noted that the amount of thymol recovered from the urine of the two series was practically the same. And it has also been remarked that no thymol could be found in the fæces.

(b) A number of convicts were then treated with our maximum dose of thymol—60 grains—administered in one portion, and the magnesium sulphate purge was given (a) two hours after, (b) one hour and a half after, (c) one hour after, (d) half an hour after treatment, (e) together with the drug. In no case was thymol detected in the stools. And in this connection (Table VIII) experiments Nos. 7 and 8 are certainly instructive as the bowels had begun to move freely three quarters of an hour after treatment, and had been voided in less than two hours. The urine showed the same average percentage of recovery (22·0 per cent) as in series 6 of Table VII.

4. *The hookworms and the elimination of thymol.*—Could the ingestion of thymol by the hookworms then be responsible for the disappearance of at least a portion of the drug? If so, for a given dosage the amount of thymol recovered ought, to some extent at least, to be inversely proportional to the number of worms expelled. The results figured in Table IX do not justify this supposition.

TABLE IX.

*Showing the amount of thymol recovered and the number of hookworms per case.*

DOSE = 10 GRAINS.		DOSE = 20 GRAINS.		DOSE = 30 GRAINS.	
Thymol recovered in grains.	Hookworms expelled.	Thymol recovered in grains.	Hookworms expelled.	Thymol recovered in grains.	Hookworms expelled.
2.11	29	5.92	116	6.97	97
4.18	16	3.84	75	4.30	63
6.74	7	5.61	39	12.48	63
6.97	0	4.59	2	4.48	3
2.27	0	5.69	1	6.37	2

DOSE = 40 GRAINS.		DOSE = 50 GRAINS.		DOSE = 60 GRAINS.	
Thymol recovered in grains.	Hookworms expelled.	Thymol recovered in grains.	Hookworms expelled.	Thymol recovered in grains.	Hookworms expelled.
9.66	170	7.19	322	15.58	216
6.12	140	15.43	186	10.98	116
9.21	89	5.58	65	16.66	79
5.52	6	7.87	5	15.75	3
6.00	4	10.74	2	10.37	2

Hookworms were collected from the faeces after treatment with thymol, thoroughly washed, and examined for the drug. No free thymol could be recovered from them.

5. *The person treated and the elimination of thymol.*—In 116 cases, all males, the age and the weight of the convict were recorded and were found to vary from 19 to 60 years, and from 84 to 143 lb., respectively. Age and body-weight do not in any way influence the absorption and elimination of the drug.

6. *The absorption of thymol and its toxicity.*—In the course of our experimentation thymol has proved throughout a very safe drug and we never had to deplore any untoward accident. The convict who eliminated 19.47 grains of thymol exhibited no more toxic symptoms than the rest.

7. *Thymolated urine.*—The colour of the urine after thymol treatment varied from yellow to dark-brown depending not only on the dose administered but also on the volume passed during the 24 hours. Its

odour was characteristic, but did not recall that of thyme. Its reaction was acid. The presence of thymol glycuronate prevented neither decomposition, nor the precipitation of urates or phosphates.

As a rule, the density was normal, and so also the volume, whenever the convict could be persuaded that drinking freely after the treatment would do him no harm; the average volumes as shown in Tables VI and VII are a proof that our convincing powers did not go a very long way.

Mono- and di-phenols were invariably present. Indican was generally absent.

154 cases were examined for albumin and no albuminuria was found to have occurred.

8. *Discussion of results.*—(i) From 20 to 43 per cent of the thymol administered per os to healthy adults was recovered from the urine, and none from the fæces.

These results are in accord with the work done by Tauber<sup>(2)</sup>, Schaffer<sup>(1)</sup>, Hanzlik and Sellman<sup>(18)</sup>, and De Jonge<sup>(8)</sup> upon phenol, and by Seidell upon thymol. It is, moreover, interesting to note that, quite recently (1918), Heintz Harmann and Loro Zila<sup>(46)</sup> have found that, at most, 40 per cent of administered quinine is excreted in the urine and fæces,—that the rest is not deposited in the organs, and must, therefore, have been destroyed. Blum (1872)<sup>(5)</sup> asserts that most of the unabsorbed thymol leaves the intestine by way of the fæces; while Seidell (1912) points to its presence in traces only.

(ii) All recoverable thymol was eliminated within the 24 hours which followed the treatment, and the after-purge was found to have no appreciable effect on the amount excreted.

These results, together with the fact that no thymol could be recovered from fæces voided less than one hour after treatment, would seem to imply that the drug is very rapidly absorbed in the system. The necessity of the after-purge becomes, therefore, questionable. In this very rapid absorption might also be found the reason why Bozzolo, Sandwith, and others have successfully used alcoholics per os during treatment with thymol. No one can fail to see the far-reaching importance of such conclusions for, in the absence of a purge and of restrictions on the nature of the drink and food after treatment, thymol medication is bound to find favour with the masses.

(iii) The amount of thymol recovered through the kidneys was proportional to the quantity ingested, and never did severe symptoms of toxicity occur.

(iv) The amount of thymol eliminated depended on the quantity administered and not on the mode of administration, and the number of hookworms expelled was not proportional to that amount.

It would appear from these results (1) that the tedious division of the treatment into three doses is really not necessary, and (2) that, when absorbed, thymol has no therapeutic value in the treatment of hookworm disease. In this case the therapeutic value of thymol would be confined entirely to its action on the worms in the intestinal tract. The one-dose treatment ought consequently to be more efficacious since, again admitting the absorption to be very rapid, the number of worms reached by the drug is likely to be greater.

(v) There was practically no effect on the urine.

The same had already been recorded by the ship surgeons of the Pacific Mail Steamship Company (1916)<sup>(39)</sup>.

#### THE RATIONALE OF THYMOL TREATMENT WITH REFERENCE TO THE REMOVAL OF HOOKWORMS.

1. *The dosage.*—In this part of our investigation our first concern was to ascertain, as definitely as possible, the maximum vermicide dose of thymol to be administered in cases of hookworm infection among adult males. Our experiments were accordingly carried out on adult and apparently healthy male prisoners whom, as already mentioned, we divided into twelve batches to be treated with different dosages of 10, 20, 30, 40, 50, and 60 grains of thymol. Six of the groups were given the dose in three portions at hourly intervals, while the other six received it in one single portion. In all cases the thymol was finely and freshly ground with its own weight of sugar so as to prevent lumping. The alimentary canal had previously been prepared by a purge. In all cases the test treatment was followed by a purge administered two or three hours after the last portion of thymol.

As 60 grains thymol in hourly 20-grain doses (standard treatment) had proved the most powerful anthelmintic at our command, it was used in all subsequent treatments to ascertain the total number of worms in each case.

A 10-grain dosage was fixed upon as the starting sub-maximal dose, in order to be able to follow both the possible toxic symptoms in the host and the hookworm removal with subsequent increasing dosages.

The stools passed after the treatment were collected, washed, and sifted through a wire-gauze sieve with 40 meshes to an inch. The residue



was thrown in black-bottomed Petri dishes and the worms picked out, classified and counted. In this way we examined all the stools for five days after a treatment or until no hookworms had been found for two consecutive days. The 'standard treatment' was repeated at intervals of 10 or more days as long as hookworms or their ova were found in the faeces. The sum-total of hookworms passed in the several treatments represents the total hookworm content of the person or group of persons treated.

TABLE X.

*Number of hookworms removed by one 'test treatment' of thymol in three portions (with pre- and after-purge).*

Experiment No.	Test treatment.	No. of cases treated.		HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS RECOVERED WITH 'TEST TREATMENT.'		
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.
1	10 grains in three portions.	10	Test treatment .	1	27	28	16.5	22.6	22.4
			Subsequent treatments.	5	92	97			
			Total Hookworms	6	119	125			
2	20 ditto	12	Test treatment .	8	203	211	32.0	55.6	54.1
			Subsequent treatments.	17	162	179			
			Total Hookworms	25	365	390			
3	30 ditto	12	Test treatment .	30	240	270	50.8	79.9	75.0
			Subsequent treatments.	29	61	90			
			Total Hookworms	59	301	360			
4	40 ditto	11	Test treatment .	18	251	269	48.5	93.0	87.0
			Subsequent treatments.	19	21	40			
			Total Hookworms	37	272	309			
5	50 ditto	10	Test treatment .	27	158	185	84.3	96.3	94.3
			Subsequent treatments.	5	6	11			
			Total Hookworms	32	164	196			
6	60 ditto	374	Test treatment .	877	11,907	12,784	89.7	96.3	95.8
			Subsequent treatments.	100	539	639			
			Total Hookworms	977	12,446	13,423			

TABLE XI.

Number of hookworms removed by one 'test treatment' of thymol in one portion (with pre- and after-purge).

Experiment No.	Test treatment.	No. of cases treated.	—	HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS RECOVERED WITH 'TEST TREATMENT.'		
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.
1	10 grains in one portion.	16	Test treatment .	4	139	143	14·8	31·3	30·4
			Subsequent treatments.	23	304	327			
			Total Hookworms	27	443	470			
2	20 ditto	17	Test treatment .	6	657	663	37·5	71·8	71·2
			Subsequent treatments.	10	258	268			
			Total Hookworms	16	915	931			
3	30 ditto	13	Test treatment .	28	382	410	84·8	95·5	94·2
			Subsequent treatments.	5	17	22			
			Total Hookworms	33	399	432			
4	40 ditto	12	Test treatment .	64	848	912	88·8	94·9	94·5
			Subsequent treatments.	8	45	53			
			Total Hookworms	72	893	965			
5	50 ditto	13	Test treatment .	37	596	633	90·0	97·0	96·6
			Subsequent treatments.	4	18	22			
			Total Hookworms	41	614	655			
6	60 ditto	19	Test treatment .	25	496	521	96·1	99·6	99·4
			Subsequent treatments.	1	2	3			
			Total Hookworms	26	498	524			

The results as figured in Tables X and XI show that :—

(a) Whether the drug be administered in one portion or in three, its anthelmintic effect increases steadily with the dosage.

(b) For the same dosage the anthelmintic action of the drug is more marked in the case of the one-dose treatment than in the case of the three-dose treatment.

(c) 60 grains thymol in one dose may be considered as the maximal dosage in case of hookworm infection among adult males,—whether the infection be due to *Ankylostoma duodenale* or *Necator americanus*.

(d) *A. duodenale* is more thymol-resistant than *N. americanus*.

2. *Thymol as a vermicide.*—Thymol is a powerful toxic vermicide for hookworms and acts as a direct poison to them. The worms expelled are often contorted and out of shape and those passed on the third day show evident signs of decomposition.

Ascarids also yield to thymol and both *Ascaris* and *Trichiuris* are to be found in the stools after treatment with the drug. When thymol was administered in 60-grain doses divided in three portions, 22.1 per cent of the *Ascaris* and 4.9 per cent of the *Trichiuris* were removed from 59 and 41 persons infected, respectively.

3. *Purging as an aid to thymol treatment.*—

(a) 13 cases were given 50 grains thymol in one portion preceded and followed by a purge. All cases had from two to five fluid motions on the first day and passed worms.

(b) 93 cases were treated with 60 grains thymol in three portions without any pre-purge. All cases had from two to five liquid motions on the first day and passed worms.

(c) Following the ordinary routine, 59 cases were treated with 50 grains thymol in one portion but no after-purge was administered. It was found that the hookworms were expelled naturally within the three days which followed the treatment. All cases but one had from one to three free fluid motions on the first day. The other case passed the worms on the second day.

(d) 102 cases were treated with 50 grains thymol in one portion and received no purge, either before or after the treatment. 95 cases had from one to three semi-solid motions on the first day. The other 7 cases passed the worms on the second or the third day.

(e) Whatever the method of treatment adopted, ascarids were mostly expelled on the second day.

4. *Thymol as a poison to the host.*—No serious toxic symptoms were noted by us even with the highest dose.

5. *Discussion of results.*—

(i) There is a gradual improvement in efficiency with increase of dosage.

This had already been noted by Darling, Barber and Hacker, who experimented with 40 and 60 grains in two portions at two hours' interval and 90, 120, and 180 grains administered in three portions at hourly intervals.

(ii) For the same dosage the one-dose treatment is more efficient than the two- or three-dose treatment (Tables XII and XIII).

*administered, as recorded by various authors.*

Authors	50 GRAINS.		60 GRAINS.			75 grains in five portions : 15 grains every hour.	90 grains in three portions.	120 grains in three portions.	180 grains in three portions.
	One portion.	Three portions.	One portion.	Three portions.	Two portions.	One portion.			
1. Ashford and	—	—	76·85 40 cases.	—	—	—	—	—	—
2. Burton Nicc	—	—	—	—	—	—	97·87 10 cases.	—	—
3. Schuffner at voort (29)	—	—	—	—	—	83·0 153 cases	—	—	—
4. Darling, B. Hacker (43)	—	—	—	88·6 10 cases.	—	—	97·8 16 cases.	90·6 8 cases.	97·0 5 cases.
5. Caius and M <sub>2</sub>	94·5 cases.	94·3 10 cases.	96·6 13 cases.	95·8 374 cases.	99·4 19 cases.	—	—	—	—

TABLE XII.

*Percentage of hookworms expelled after 'test treatment' with varying dosages of thymol, differently administered, as recorded by various authors.*

Authors.	PEOPLE EXPERIMENTED ON.			HOOKWORM INFECTION.			30 GRAINS.			40 GRAINS.			50 GRAINS.		60 GRAINS.			75 grains in five portions : 15 grains every hour.	90 grains in three portions.	120 grains in three portions.	180 grains in three portions.
	Nationality.	Sex.	Age.	'Health.'	Infection.	Species formula.	Three portions.	Two portions.	One portion.	Three portions.	Two portions.	One portion.	Three portions.	One portion.	Three portions.	Two portions.	One portion.				
1. Ashford and King <sup>(12)</sup>	?	?	?	Hospital cases	1,000 hook- worms per case.	?	—	—	—	—	—	—	—	—	76.85 40 cases.	—	—	—	—	—	—
2. Burton Nicol <sup>(24)</sup>	Indians	Males	Adults	Coolies	?	?	—	—	—	—	—	—	—	—	—	—	—	—	97.87 10 cases.	—	—
3. Schuffner and Ver- voort <sup>(25)</sup>	?	?	?	?	29 hookworms per case.	?	—	—	—	—	—	—	—	—	—	—	—	83.0 133 cases	—	—	—
4. Darling, Barber and Hacker <sup>(46)</sup>	1. Malay natives. 2. Chinese . 3. Malabaris . 4. Pathans . 5. Sikhs .	Males	Adults	Prisoners	58 hookworms per case.	88 Necator to 12 Ankylostoma.	—	—	—	—	83.4 33 cases.	—	—	—	—	88.6 10 cases.	—	—	97.8 16 cases.	90.6 8 cases.	97.0 5 cases.
5. Cais and Bhaskar	Tamil Indians	Males	Adults (20-50 years of age).	Prisoners	55 hookworms per case.	94 Necator to 6 Ankylostoma.	75.0 12 cases.	88.1 6 cases.	94.2 13 cases.	87.0 11 cases.	—	94.5 12 cases.	94.3 10 cases.	96.6 13 cases.	95.8 374 cases.	—	99.4 19 cases.	—	—	—	—

TABLE XIII.

*Anthelmintic action of 30 grains dosage of thymol.*

Test treatment.	No. of cases treated.		HOOKWORMS REMOVED.			PERCENTAGE RECOVERED AFTER "TEST TREATMENT."		
			duodenale.	N. americanus.	Both.	A. duodenale.	N. americanus.	Both.
In three portions at hourly intervals.	12	Test treatment .	30	240	270	50·8	79·9	75·0
		Subsequent treatments.	29	61	90			
		Total Hookworms	59	301	360			
In two portions at hourly intervals.	6	Test treatment .	5	293	298	50·0	89·3	88·1
		Subsequent treatments.	5	35	40			
		Total Hookworms	10	328	338			
One-portion treatment.	13	Test treatment .	28	382	410	84·8	95·5	94·2
		Subsequent treatments.	5	17	22			
		Total Hookworms	33	399	432			

We believe we are the first to have noticed this fact.

It is interesting to mark here that 30 grains thymol administered in one portion is as effective as 50 grains given in three portions (Tables X and XI).

(iii) The 60 grains dose administered in one portion may be taken as representing the maximal vermifugal dosage.

With this dosage we have reached the largest worm removal associated with a toxicity below the host's limit of tolerance to the drug—a limit which we had no necessity to work out. Darling, Barber and Hacker reached the largest worm removal with 90 grains in three doses and declared that when the dose is increased beyond this, there is no improvement (Table XII). In this series 15·8 per cent of their patients vomited and the series treated with higher doses showed that the limit of tolerance to the drug had been exceeded. In view of such results we did not feel justified in trying higher doses than 60 grains.

(iv) Whatever the mode of administration and the dosage, *Ankylostoma duodenale* is more thymol-resistant than *Necator americanus*.

It has been generally surmised that *Ankylostomum* is more resistant than *Necator* to the action of vermicides.

(v) Thymol is a powerful anthelmintic.

It is unanimously admitted that thymol acts as a potent poison to hookworms.

In our opinion it is an equally powerful ascaricide. Darling, Barber and Hacker mention that 45 grains thymol removed 20 per cent, and 90 grains 32·7 per cent, of the ascarids present, and we have found that with 60 grains 27 per cent are expelled. Thus, as is the case with hookworms, the percentage of ascarids removed is proportional to the amount of thymol ingested and the relatively small figures are accounted for by the position of these worms in a lower part of the intestinal tract which the drug cannot reach in any high degree of concentration owing to the rapidity with which it is absorbed.

(vi) Thymol has a vermifugal action.

The number and nature of the motions obtained with 50 grains of thymol in the absence of a purgative show that the drug itself is able to stimulate the intestine and promote evacuation, one of its well-known physiological properties.

The pre-purge was already being omitted in severe ankylostomiasis or in constitutional diseases attended with great debility; and we understand that many workers have suppressed it, altogether, from their routine treatment. That the pre-purge does not appreciably increase the efficiency of the treatment may be gathered from the results shown in Table XIV [(a) and (b)].



TABLE XIV.

*Anthelmintic action of thymol with or without purging.*

Test treatment.	No. of cases treated.		HOOKWORMS REMOVED.			PERCENTAGE RECOVERED AFTER 'TEST TREATMENT.'		
			A. duodenale.	N. americanus.	N. A. duodenale and americanus.	A. duodenale.	N. americanus.	N. A. duodenale and americanus.
(a) Thymol 60 grains, in 3 portions, with a pre- and after purge.	374	Test treatment .	877	11,907	12,784	89.7	96.3	95.8
		Subsequent treatments.	100	539	639			
		Total Hookworms	977	12,446	13,423			
(b) Thymol 60 grains, in 3 portions; no pre-purge but after-purge given.	93	Test treatment .	178	2,808	2,986	89.0	96.3	95.9
		Subsequent treatments.	21	105	126			
		Total Hookworms	199	2,913	3,112			
(c) Thymol 50 grains, in one portion, with a pre- and after-purge.	13	Test treatment .	37	596	633	90.0	97.0	96.6
		Subsequent treatments.	4	18	22			
		Total Hookworms	41	614	655			
(d) Thymol 50 grains, in one portion, with a pre-purge but no after-purge.	59	Test treatment .	138	1,849	1,987	63.3	93.9	90.8
		Subsequent treatments.	80	120	200			
		Total Hookworms	218	1,969	2,187			
(e) Thymol 50 grains, without any purge.	52	Test treatment .	21	470	491	?	?	?
		Subsequent treatments.	29	100	129			
		Total Hookworms	50	570	620			
(f) Thymol 50 grains, without any purge.	50	Test treatment .	11	105	116	?	?	?
		Subsequent treatments.	14	38	52			
		Total Hookworms	25	143	168			

We have already noted that the rapid absorption of thymol raised doubts as to the necessity of the after-purge; that fact taken together with the vermifugal action of the drug, recorded here, leads to the natural inference that the after-purge may be dispensed with.

It may, however, be asked whether the suppression of the after-purge will not result in some serious inconvenience to the host? Unaided by a purgative the vermifugal action of thymol might be slow enough to allow of the partial decomposition or digestion of the dead worms in the intestine, and toxic symptoms might develop in consequence. As many as 161 of our cases received no after-purge and nevertheless showed no toxæmia. The patient, on the other hand, felt all the better in the absence of the weakening effect of a purge and readily went on with his work. The 2 to 5 lb. loss of weight which generally followed the ordinary treatment did not occur.

The after-purge incidentally helps in collecting the worms and calculating the hookworm content which has been taken as the basis for one of the methods used in the determination of the anthelmintic value of drugs. We collected the worms passed by 59 of our 'no after-purge' cases and calculated the percentage of worms recovered after the 'test treatment.' The results show [Table XIV (c) and (d)] that most of the hookworms were recovered from the fæces. This we ascribe to the pre-purge whose continued action may be inferred from the fluidity of the stools. On the other hand, our 102 'no purge' cases had semi-solid motions and passed a relatively small number of worms; 52 of these were new admissions into the jail [Table XIV (e)], while the other 50 had served periods of 1 to 7 years [Table XIV (f)].

(vii) Thymol is a safe drug.

We have shown that with doses up to 60 grains—which dosage represents the maximal vermucidal dosage—no toxic symptoms develop in the host and that the elimination of the after-purge is not followed by toxæmia.

Both Bozzolo and Sandwith, even with higher doses, administered alcoholic drinks freely during medication with thymol, and Ashford allowed his patients to take the drug home with them knowing that they undoubtedly would drink rum afterwards. According to Osler<sup>(14)</sup>, the practice has been to use castor oil as the last aperient, a practice followed by Stiles, Schuffner and Vervoort. It thus appears that the danger of poisoning due to the use of thymol solvents has been exaggerated.

ministered, as recorded by various authors.

Authors.	40 GRAINS.			50 GRAINS.		60 GRAINS.		75 grains in three portions.	90 grains in three portions.	Results after two treatments with 60 grains in three portions.
	One portion.	Three portions.	One portion.	Three portions.	One portion.	Three portions.	One portion.			
1. Wyler ( <sup>59</sup> )	—	53.0 17 cases.	—	—	—	70.3 47 cases.	—	—	89.5 57 cases.	—
2. Burton Nicol ( <sup>2</sup> )	—	—	—	—	—	—	—	43.8 75 cases.	63.0 197 cases.	—
—	—	—	—	—	—	69.0 26 cases.	—	72.7 11 cases.	—	—
3. Macallan ( <sup>54</sup> )	—	—	—	—	—	70.0 cases ?	—	—	—	—
—	—	—	—	—	—	50 to 50 % cases ?	—	—	—	—
4. Bailey ( <sup>57</sup> )	—	—	—	—	—	59.0 cases ?	—	—	—	—
5. Rockefeller Co sion (1916)	—	—	—	—	—	—	—	—	—	45.9* 170 cases.
—	—	—	—	—	—	—	—	—	—	51.0† cases ?
6. Barnes ( <sup>41</sup> , <sup>42</sup> )	—	—	—	—	—	80.4 197 cases.	—	—	—	—
7. Fergusson ( <sup>4</sup> )	—	—	—	—	—	—	—	—	—	—
8. Cains and Mhas	46.1 3 cases.	36.3 11 cases.	25.0 12 cases.	50.0 10 cases.	64.3 13 cases.	64.4 374 cases.	88.8 19 cases.	—	—	—

TABLE XV.

*Percentages of cures obtained with one 'test treatment' with varying doses of thymol, differently administered, as recorded by various authors.*

Authors.	Nationality.	PEOPLE EXPERIMENTED ON.			HOOKWORM INFECTION.				30 GRAINS.			40 GRAINS.		50 GRAINS.		60 GRAINS.		75 grains in three portions.	90 grains in three portions.	Results after two treatments with 60 grains in three portions.
		Sex.	Age.	Health.	Infection.	Species formula.	Method of diagnosing hookworm ova infection.	10 grains every night until 100 doses are taken.	Three portions.	Two portions.	One portion.	Three portions.	One portion.	Three portions.	One portion.	Three portions.	One portion.			
1. Wyler ( <sup>1</sup> )	?	Males	Adults	Prisoners	?	?	?	—	—	—	—	53.0 17 cases.	—	—	—	70.3 47 cases.	—	—	89.5 57 cases.	—
2. Burton Nicol ( <sup>2</sup> )	Indians	Males	Adults	Coolies	?	?	?	—	—	—	—	—	—	—	—	—	—	43.8 75 cases.	63.0 197 cases.	—
	Indians	Females	Adults	Coolies	?	?	?	—	—	—	—	—	—	—	—	68.0 26 cases.	—	72.7 11 cases.	—	—
3. Macallan ( <sup>3</sup> )	Egyptians	?	?	?	Light	?	?	—	—	—	—	—	—	—	—	70.0 cases ?	—	—	—	—
	Egyptians	?	?	?	Heavy	?	?	—	—	—	—	—	—	—	—	30 to 50 % cases ?	—	—	—	—
4. Bailey ( <sup>4</sup> )	Nicaragua	?	?	?	?	?	?	—	—	—	—	—	—	—	—	59.0 cases ?	—	—	—	—
5. Rockefeller Commission (1916)	Trinidad	?	?	?	?	?	?	—	—	—	—	—	—	—	—	—	—	—	—	45.9* 170 cases
	Trinidad	?	?	?	?	?	?	—	—	—	—	—	—	—	—	—	—	—	—	51.0† cases ?
6. Barnes ( <sup>41</sup> , <sup>42</sup> )	Siamese	?	?	?	?	?	Barber's specific gravity method.	—	—	—	—	—	—	—	—	80.4 197 cases.	—	—	—	—
7. Fergusson ( <sup>6</sup> )	British Guiana	?	?	?	?	?	?	100% (all cured in 9 months).	—	—	—	—	—	—	—	—	—	—	—	—
8. Cairns and Mhaakar	Tamilians	Males	Adults years of 20-50 ages.	Prisoners	55 hookworms per case.	94 Necator to 6 Ankylostoma.	Howard's Centrifugal method.	—	16.1 12 cases.	0 6 cases.	46.1 13 cases.	36.3 11 cases.	25.0 12 cases.	50.0 10 cases.	64.3 13 cases.	64.4 374 cases.	88.8 19 cases.	—	—	—

\* Thymol and sugar.

† Thymol and soda bicarb.

We have, therefore, treated 102 cases without putting any restriction on the nature and the quantity of the food either before or after treatment, and the convicts have been all the fitter for it.\*

#### THE EFFICIENCY OF THE THYMOL TREATMENT.

If the powerful anthelmintic properties of thymol are universally recognised, opinions still vary as to the efficiency of the treatment. This want of consensus is not only due to the springing up of new drugs, but also to the difference in the results obtained.

This difference in the results may, first of all, be accounted for by different methods having been adopted for the determination of the efficiency. Three of the methods may be mentioned here :—(1) method dependent on the percentage of the hookworms removed with one test treatment ; (2) method resting on the percentage of cures obtained with one test treatment ; (3) method based on the average number of treatments required to cure a group of infected persons. As this last method is chiefly intended for the comparison of the degrees of efficiency noted with different drugs, it need not be discussed at this stage.

Tables XI and XV record the results arrived at by different workers. It is seen that the results are not solely subject to the action of the drug, but also largely depend on many complicating factors which ought not to be ignored if the results are to be compared.

Thus efficiency will vary with :—

(i) The dose administered and the mode of administration, which both influence the degree of concentration with which the drug reaches the parasites, and are both conditioned by the apparent age, sex, and health of the host.

(ii) The number and species of hookworms harboured ; the species, which is to decide with what readiness the parasites are to yield to the drug, mostly depends on the tract of land inhabited by the host and the ethnic stock he belongs to.

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\* A type case treated on the above lines in private showed no toxic symptoms due to thymol or to the decomposition of dead worms. The patient was a woman, aged 22 years, of slight build, multipara, with pregnancy of 7 months, advanced anæmia, oedema of the whole body which started four months back in the legs, difficulty of breathing, pain in abdomen and impaired appetite ; hæmic murmurs in the heart, pulse feeble and 100 per minute ; hæmoglobin 10 per cent, no albumin in urine ; examination of fæces showed 10 hookworm ova per microscope field. She was treated, without any previous preparation, with thymol 40 grains according to her apparent age. She received no after-purge and was allowed her usual diet. She had five motions during the day of treatment and experienced no inconvenience beyond giddiness.

If all these points are taken into account, results obtained by determining the percentage of hookworms removed with the one test treatment, must represent the degree of efficiency of the treatment, with sufficient accuracy to allow comparison.

Not so the results based upon a determination of the percentage of cures so long as 'cure' is understood to mean the absence of hookworm ova from the faeces 10 days or more after a treatment. For, as (Clayton Lane (<sup>47</sup>) most appropriately remarks when discussing the diagnosis of hookworm infection based on the recognition of the hookworm ova in the faeces, this method has its fallacies: '(1) Ova of the hookworm type are common to the hookworm and to a number of presumably rare parasites of man; (2) hookworms may be present in the bowel and yet no hookworm ova may be found in the examination of the stools; (3) although ova are present in the stools they are not found by microscopic examination because they are present in relatively small numbers, and a sufficiency of stool has not been examined; (4) the relative values of different methods for the detection of hookworm ova appear at present to rest far more on personal opinions than should be the case in a matter of this importance and there appears to be no common standard by means of which they can be compared one with another.'

Another complicating factor which bears not only on the 'cures' but also on the hookworm content arises from the life-history of the hookworm. It is well known that the parasite takes from six to seven weeks to reach the intestine after infecting the host and there is no means to tell whether the worms passed with subsequent treatments were really present at the time of the test treatment.

We have relied on the hookworm removal for determining the efficiency of various anthelmintics, as the method, with all its desiderata, is still the best at our command.

Table XII shows that in cases of hookworm infection thymol treatment is decidedly efficient.

#### CONCLUSIONS.

1. Thymol is a solid drug of constant chemical composition, not deteriorating with age, and easily obtained in the pure state. Its dosage is thus easy and certain.

2. Free thymol is very soon eliminated from the system, the danger of poisoning for the host thus being greatly diminished; and its quick disappearance from the intestine renders the after-purge and any

restriction on the nature of the food unnecessary. Up to a 60-grain dosage thymol is a safe drug.

3. Thymol is a powerful vermicide acting both on *Ankylostoma* and *Necator*. Any dose from 30 to 60 grains administered in one portion will prove effective.

4. Thymol has mild vermifugal properties.

Treatment with thymol may then be reduced to the simple ingestion of the drug in proper dosage.

This treatment not interfering with the daily occupations of the individual is likely to find favour with the masses and, therefore, meets the condition of a light and wide infection. The convicts of the Trichinopoly Jail are fairly representative of the civil population—peasants, coolies, artisans, etc., of the Madras Presidency.

The elimination of the purges and the absence of medical supervision help materially in bringing down the cost of treatment.

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IDENTIFICATION OF THREE STRAINS OF  
TRYPANOSOMES FROM CASES OF  
SLEEPING SICKNESS CONTRACTED  
IN PORTUGUESE EAST AFRICA  
WITH TRYPANOSOMA  
RHODESIENSE.

BY

CAPTAIN (TEMPY.-MAJOR) T. A. HUGHES, M.B., I.M.S.

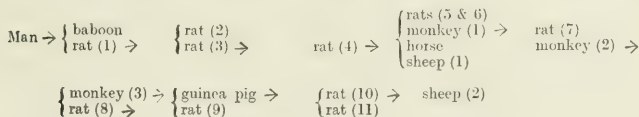
[Received for publication, February 18, 1919.]

THE cases from which the strains were obtained contracted the disease in the hinterland of Port Amelia, about 160-180 miles south of the Rovuma, a district infested with *Glossina morsitans*, the transmitter of *Trypanosoma rhodesiense*. *Glossina palpalis*, the carrier of *Trypanosoma gambiense*, is said not to occur in Portuguese East Africa (1). When they arrived in Daressalaam attempts were made to confirm a diagnosis of rhodesiense-infection by the production in sub-inoculated animals of the typical posterior nuclear forms described by Stephens and Fantham (2). Apart from this morphological peculiarity and the fact that it is carried by *Glossina morsitans*, *Trypanosoma rhodesiense* differs from *T. gambiense* in that it is more virulent, is atoxyl-resistant, produces oedema of the face and keratitis in sheep and goats (3) and is susceptible to the action of human and baboon sera (4). The two trypanosomes can also be differentiated by cross-immunity experiments. The animals available for inoculation were field rats, monkeys (*Cercocebus albicularis*), rabbits (two), sheep (two), a goat, a horse, and a baboon (*Papio cynocephalus*). Rats were examined for *T. lewisi* before inoculation and any found infected were not used in the experiments.

Stephens and Fantham (*loc. cit.*) observed posterior nuclear forms in rats infected with *Trypanosoma rhodesiense* on the 5th or 6th day of the disease. They increased in number up to the 11th day, when they

formed as many as 6 per cent of the parasites. Nuclear displacement was observed only in the short 'stumpy' forms, and at periods when the animal's blood contained numerous parasites. Yorke (6) observed posterior nuclear forms in rats, mice, monkeys, guinea-pigs, rabbits, dogs and horses. Blacklock (6) found that these forms increased in number in rats relatively to the other forms of the parasite as the disease progressed, and that those with the nucleus near the centre appeared first, those with the nucleus near the blepharoplast last, while forms with the nucleus in an intermediate position appeared at intervening periods. Wenyon and Hanschell (7), working with three strains of *T. rhodesiense*, found that the percentage of posterior nuclear forms varied in different strains and that these forms were more numerous in the first than in succeeding passages in the rat. Beck and Week (8) described trypanosomes from the Rovuma district, 'German East Africa', which they considered differed morphologically from both *T. rhodesiense* and *T. gambiense*. They never observed transposition of the nuclei on sub-inoculation. Taute (9), however, previously showed that certain trypanosomes from the Rovuma district did correspond to *T. rhodesiense*. Beven and Millington (10) failed to find posterior nuclear forms in animals infected from a patient who contracted trypanosomiasis in Northern Rhodesia. The strain did not produce the typical oedema of the face in sheep. Kinghorn, however, found posterior forms in animals infected with this strain.

The first strain investigated (Mn) was carried through nine passages as follows :—



All the inoculations were made subcutaneously. Details are given in the appendix.

The infection proved fatal to all rats and monkeys and to the guinea-pig. The incubation period in rats varied from 4 to 8 days—average 6—and the period to death varied from 6 to 27 days with an average of 12.6. In the monkeys the incubation periods were 6, 6, and 7 days and death occurred 12, 7, and 12 days, respectively, after inoculation. The guinea-pig was first examined on the 11th day, when it had trypanosomes in its blood. It was alive 31 days after inoculation, its blood still containing parasites.

At this period I left Daressalaam, but I have since been informed that it died subsequently. The horse was not under direct observation. It was alive and running an irregular temperature 62 days after inoculation, its blood containing trypanosomes. It subsequently became very ill and was slaughtered. Sheep (1) had parasites in its blood on the 11th day (first examination) and is still alive (six months after inoculation). Trypanosomes, in small numbers, were found in its blood for about 10 days, after which they were not found. At present the animal is very ill and emaciated. There is well-marked facial œdema and some cloudiness of the corneæ. Sheep (2) was inoculated two days before I left Daressalaam. Its blood has been fairly regularly examined but trypanosomes were never found. At present (4 months after inoculation) there is considerable œdema of the face and the animal is very thin. The corneæ are slightly clouded. Both sheep will probably soon succumb to the disease.\*

The bloods of all these animals were repeatedly—in most cases, daily—examined for the presence of the posterior nuclear forms of the trypanosome. Both thick and thin films were used, stained with Azur II and Eosin. These forms were found only in the case of monkey (1) in the fourth passage. This animal showed them the day before death when the infection became very intense, the oil-immersion field (1 12" lens and Zeiss II ocular) containing as many as 30 to 50 parasites. Forms with the nucleus posterior to the centre numbered 0.8 per cent (1,000 counted). Of these only half, *i.e.*, 0.4 per cent, had the nucleus in the posterior third. None were observed with the nucleus posterior to the blepharoplast. Figures 1, 2 and 3 show the maximum nuclear displacement observed.

The second strain (Mk) was passed from the patient into a monkey. This animal had trypanosomes in its blood on the 6th day and lived for 30 days after inoculation. The infection was at first intense—about 20 to 30 parasites to the microscopic field. On the seventh day posterior nuclear forms appeared. As in the case of the first strain they numbered 0.8 per cent (1,000 counted) and of these only half, *i.e.*, 0.4 per cent, had the nucleus in the posterior third (Fig. 14). None had nucleus behind the blepharoplast. The infection then became less intense and took on an undulating character. No posterior nuclear forms were subsequently seen. Four rats, a guinea-pig and another monkey were inoculated from this animal. The incubation periods were:—rats 4 to 7 days—average 5.8;

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\* Sheep (1) died on 23-1-19, and sheep (2) on 25-1-19.

guinea-pig 11 days (first examination); monkey 6 days. The times to death were: rats 7 to 10 days—average 8·4; guinea-pig 16 days, monkey 8 days. In none of these animals were posterior nuclear forms seen. In the third passage two rats were inoculated, which died in 10 and 7 days, respectively. Posterior nuclear forms were not found in their bloods. The following is a summary of the inoculations (all subcutaneous) made with strain 2:—

Man → monkey (1)  $\left\{ \begin{array}{l} \text{rats (1, 2, 3, \& 4)} \\ \text{guinea-pig} \\ \text{monkey (2)} \end{array} \right\} \rightarrow \text{rats (5 \& 6)}$

In Daressalaam strain 3 (L) was carried through two passages as follows:—

Man →  $\left\{ \begin{array}{l} \text{rat (1)} \\ \text{monkey} \end{array} \right\} \rightarrow \text{rats (2 \& 3). (Inoculations subcutaneous.)}$

The animals in the first passage had incubation periods of 6 days each, and both died on the 7th day. In the second passage, the incubation period was five days in each case and both rats died on the 14th day. No posterior nuclear forms were found in these animals. At a later period in Nairobi, two rabbits were inoculated intraperitoneally with this strain direct from the patient. Trypanosomes were found in both after 4 days' incubation. The rabbits rapidly lost weight and seemed very ill. The following is a summary of observations carried out on the animals.

Rabbit (1):—

- 7-12-18 .. Inoculated.
- 11-12-18 .. Parasites first seen—very few—long slender forms.
- 12-12-18 .. Parasites fairly numerous—a few 'stumpy' forms—some 'snout' forms also seen.
- 13-12-18 .. Parasites very numerous—many 'stumpy' forms—animal died during the night.

No posterior nuclear forms were seen. One thousand parasites were counted on each of the last two days.

Rabbit (2):—

- 7-12-18 .. Inoculated.
- 11-12-18 .. Parasites first seen—very few—long slender forms.
- 12-12-18 .. Parasites numerous—some 'snout' forms seen.

468 *Trypanosomes from Sleeping Sickness in Africa.*

13-12-18 .. 96,400 parasites per cmm. (counted by means of a Thoma-Zeiss haemocytometer) mostly long and intermediate forms—many showing 'snouts.'

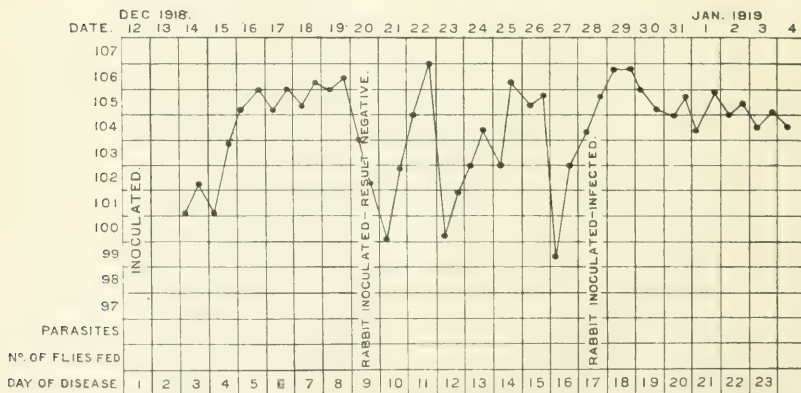
14-12-18 .. 116,250 parasites per cmm., a few 'stumpy' forms.

15-12-18 .. 140,000 parasites per cmm., 'stumpy' forms numerous—animal died in convulsions.

One thousand trypanosomes were counted on the 13th and on the 14th. No posterior nuclear forms were seen. On the 15th, 1,000 were counted from the peripheral blood, and 2,000 from the heart's blood. Three parasites with the nucleus in the posterior third were found (Fig. 17), *i.e.*, 0.1 per cent of the 3,000 counted. None were seen with the nucleus posterior to the blepharoplast.

The only symptoms presented by the animals were anæmia and emaciation. The other usual symptoms of trypanosomiasis in rabbits, *viz.*, blepharo-conjunctivitis, oedema of the head and ears, and purulent discharge from the nose and eyes, were not present; probably owing to the short course of the disease.

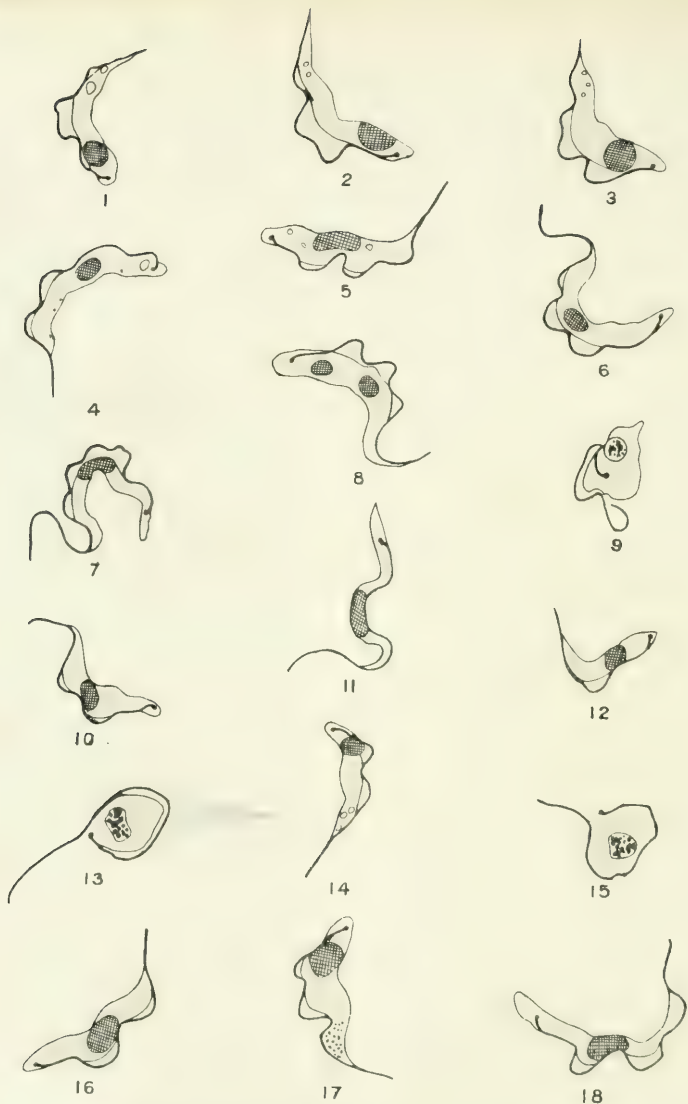
A goat was inoculated subcutaneously from rabbit (2) on 12-12-18. Its temperature chart for the time it has been under observation is reproduced. The animal lost weight almost from the outset although



Temperature Chart of goat inoculated with strain 3.



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Figs. 1 to 9—Parasites from blood of monkey (1). Strain 1

Figs. 10 to 14—Parasites from blood of monkey (1). Strain 2.

Figs. 15 to 18—Parasites from blood of rabbit (2). Strain 3.

Drawn with an Abbe camera lucida using a Zeiss IV Ocular and a Zeiss 1/12" lens

parasites were not seen in its blood until the 24th day. A rabbit inoculated subcutaneously on the 8th day with about 1 c.c. of the goat's blood did not become infected. Another rabbit similarly inoculated on the 17th day had trypanosomes in its blood on the 8th day after inoculation. When last seen (26th day) the goat was very emaciated, had some swelling of the face and a nasal discharge, and did not seem likely to live long. Posterior nuclear forms were not seen.

Posterior nuclear forms were found, therefore, in all three strains, but only in small numbers and only when the infection was very intense. On comparing blood films from the monkeys, showing posterior nuclear forms of strains 1 and 2, with films from rabbit (2) infected with strain 3, the infection in both monkeys seemed heavier than in the rabbit. None of the other animals had infections as intense as these three. Hence parasites with posteriorly dislocated nuclei appeared only when the trypanosomes numbered at least 140,000 per cmm. in the peripheral blood, and even in infections of this intensity, forms with the nucleus in the posterior third numbered only 0.4, 0.4 and 0.1 per cent, respectively, and no forms were found with the nucleus posterior to the blepharoplast. These strains are therefore characterised by the great reluctance with which they produced posterior nuclear forms.

The strains were very polymorphic, and posteriorly dislocated nuclei were observed only in the 'stumpy' forms. Protoplasmic granules and vacuoles were seen at the anterior end in many of these forms. Long slender trypanosomes were most numerous during the first day or two. Many of these had elongated posterior ends—the 'snout' forms described by Stephens and Fantham (Figs. 7, 11, and 18). In the very heavy infections, round leishmania-like forms were seen. Some of these had flagellæ (Figs. 9, 13, and 15). Measurements were made of 100 parasites from the blood of monkey (1) (first strain). They varied from  $12.72\mu$  to  $27.56\mu$  in length, the average being  $18.23\mu$ . In infected animals the parasites either underwent a periodic variation or steadily increased in number till death. In rats showing a periodic variation the times when trypanosomes were most numerous in the blood were separated by intervals of 3 or 4 days. This periodic increase and decrease in trypanosome infections was described by Fantham and J. G. Thomson<sup>(11)</sup>.

The pathogenicity of the strains for all the animals inoculated would correspond, on the whole, to that of *T. rhodesiense* (Yorke: *loc. cit.*) Strain 3 was very pathogenic for rabbits. The disease produced in

sheep by strain 1 ran a more chronic course than is usually described for rhodesiense infection in this animal. It is possible that the virulence of this trypanosome for sheep would be enhanced by passage. With the strain experimented with by Bevan and Millington—already referred to—which failed to produce typical oedema of the face in sheep, the animals succumbed in an average of 69 days. With regard to the effect on man, I learned from the medical officers in charge that the disease was severe in all cases, the prognosis bad, and that atoxyl was without effect on the course of the illness. Tatar emetic intravenously caused temporary disappearance of the parasites from the peripheral blood, but they always returned.

On the whole, the strains must from their general pathogenicity, their morphology, their atoxyl-resistance, and the fact that they came from a morsitans-infected area be classified as *Trypanosoma rhodesiense*. The great paucity of posterior nuclear forms in sub-inoculated animals shows that certain strains of this parasite may produce these forms in very small numbers and only in very intense infections. The effect of strain (1) on sheep would tend to show that the pathogenicity for certain animals may vary in different strains.

I have to express my thanks to Lieut.-Colonel H. B. Newham, C.M.G., R.A.M.C., Consultant in Tropical Disease, East Africa Force, and to Major R. Semple, O.B.E., R.A.M.C., O.C. No. 1 British Bacteriological Laboratory, East Africa Force, for many helpful suggestions.

I have also to thank Captains Sharpe, W. K. McDonald and Shaw, R.A.M.C., for permission to see the cases and make inoculations therefrom.

## APPENDIX.—INOCULATION.

STRAIN.	Date of Inoculation.	Date of first appearance of trypanosomes in blood.	Inoculation period, in days.	Date of death.	Days till death.	REMARKS.
<i>Strain 1 (Mn.).</i>						
Man.						
1st passage { baboon	7-6-18	.....	..	.....	..	Not infected.
rat (1)	7-6-18	.....	5	14-6-18	7	
2nd passage { rat (2)	13-6-18	.....	7	6-7-18	23	Slaughtered. Alive 6 months after inoculation.*
rat (3)	14-6-18	.....	4	20-6-18	6	
3rd passage { rat (4)	19-6-18	.....	..	4-7-18	15	Slaughtered. Alive 6 months after inoculation.*
rat (5)	25-6-18	.....	..	9-7-18	14	
4th passage { rat (6)	4-7-18	.....	..	31-7-18	27	Posterior nuclear forms found on 11th day.
horse	4-7-18	.....	11(?)	.....	..	
sheep (1)	4-7-18	15-7-18 (probably earlier)	..	.....	..	Slaughtered. Alive 6 months after inoculation.*
monkey (1)	4-7-18	.....	6	16-7-18	12	
5th passage { monkey (2)	10-7-18	.....	6	17-7-18	7	Slaughtered. Alive 6 months after inoculation.*
rat (7)	14-7-18	.....	6	23-7-18	9	
6th passage { monkey (3)	23-7-18	.....	7	4-8-18	12	Slaughtered. Alive 6 months after inoculation.*
rat (8) ↓	23-7-18	.....	5	31-7-18	8	

\* Died 23-1-19.

APPENDIX.—INOCULATION—*Continued.*

STRAINS.	Date of Inoculation.	Date of first appearance of trypanosomes in blood.	Inoculation period, in days.	Date of death.	Days till death.	REMARKS.	
<i>Strain 1 (Mn.)—contd.</i>							
7th passage ↓ rat (9) ↓ guinea-pig	31-7-18	.....	..	10-8-18	10	{ Alive on 3-9-18. Died later.	
	4-8-18	15-8-18 (probably earlier)	11(?)	19-11-18	107(?)		
8th passage ↓ rat (10) ↓ rat (11)	22-8-18	..	7	.....	..		
	23-8-18	31-8-18	8	.....	..		
9th passage ↓ sheep (2)	1-9-18	Alive four months later.*					
<i>Strain 2 (Mk.).</i>							
Man.							
1st passage ↓ monkey	14-7-18	..	6	14-8-18	31	Posterior nuclear forms found on 7th day.	
		20-7-18					
2nd passage ↓ rat (1) ↓ rat (2) ↓ rat (3) ↓ rat (4) ↓ monkey (2) ↓ guinea-pig	22-7-18	..	7	1-8-18	10		
	27-7-18	31-7-18	4	3-8-18	7		
	6-8-18	12-8-18	6	16-8-18	10		
	10-8-18	15-8-18	5	18-8-18	8		
	14-8-18	20-8-18	6	22-8-18	8		
3rd passage ↓ rat (5) ↓ rat (6)	4-8-18	15-8-18 (probably earlier)	11(?)	20-8-18	16		
	15-8-18	22-8-18	7	25-8-18	10		
	20-8-18	.....	..	27-8-18	7		

\* Died 25-1-19.

## APPENDIX.—INOCULATION—Concluded.

STRAIN.	Date of Inoculation.	Date of first appearance of trypanosomes in blood.	Inoculation period, in days.	Date of death.	Days till death.	REMARKS.
<i>Strain 3 (L).</i>						
Man.						
↓						
1st passage { rat (1)	18-6-18	24-6-18	6	25-6-18	7	
monkey	18-6-18	24-6-18	6	25-6-18	7	
↓						
2nd passage { rat (2)	25-6-18	30-6-18	5	9-7-18	14	
rat (3)	25-6-18	30-6-18	5	9-7-18	14	
All the above inoculations were made subcutaneously.						
<i>Further inoculations with strain 3 (L).</i>						
Man.						
↓						
Intraperi- { rabbit (1)	7-12-18	11-12-18	4	13-12-18	6	Posterior nuclear forms found on 15-12-18.
toneal. { rabbit (2)	7-12-18	11-12-18	4	15-12-18	8	
↓						
(Subcutaneous) goat	12-12-18	4-1-19	?	Alive but very ill on 6-1-19.		



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# STUDIES IN ANKYLOSTOMIASIS.

## No. IV

BY

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IN studies Nos. I to III upon Ankylostomiasis, my assistants and I found that *Chenopodium* oil, Thymol and Manson's Mixture were not so effective in sterilizing men of ankylostoma as is commonly supposed, and that, unless our work contained grave and undetected errors, the claims to cures by one to two treatments, made upon so large a scale, would not bear the test of better methods and more prolonged investigation.

Such being the case, my next investigation was to test various methods, with the object of securing one that was quick, simple, and efficacious in detecting the presence of ankylostoma ova in the stools.

I tested and compared the following methods :—

(1) Water emulsion spread upon one-third of a microscopic slide, marked off by glass pencil lines.

(2) Water emulsion thickly spread and heaped up on a marked third of a slide and, after a delay of five or more minutes for settlement, depressed under water, to get rid of the excess, by means of Colonel Clayton Lane's depressor and springs in the manner described by him in the *Indian Journal of Medical Research*, July 1918. Colonel Clayton Lane was good enough to provide me with these springs and depressors.

(3) A hydrochloric acid (33 per cent) and ether emulsion spread under a coverslip, the size of one-third of a microscope slide.

(4) An excess of the same emulsion placed upon a similar coverslip (ether dissolves glass pencil marks) and, after five minutes or more for settlement, the excess pipetted off.

(5) An excess of the same emulsion placed upon a coverslip, and the coverslip itself upon a slide, and, after a delay of five or more minutes for settlement, depressing both under water with Colonel Clayton Lane's depressor to permit the excess to wash off.

The emulsions were made from one scoopful of fæces taken from five different parts of the stool. The scoops were made in the bazaar and were of similar size.

Twenty specimens were tested by each method, the total ova being counted. Two methods were compared at one time. It would have been better to have tested all five methods at the same time. My reason for limiting the comparison was that I had to snatch opportunities for this work from the medical and clerical work of a large military hospital, and it takes less time to prepare and test two specimens than five. Two coverslips or slides were thus compared, the ova on each counted and the lower count divided into the higher. The figures thus arrived at, in the twenty comparisons, were added together and divided by 20. The results were as follows :—

Method (1) was to method (2) as 1 : 2·37

Method (2) was to method (3) as 1 : 6·06

Method (2) was to method (4) as 1 : 12·76

Method (2) was to method (5) as 1 : 11·42

Method (3) was to method (4) as 1 : 6·04

Method (3) was to method (5) as 1 : 7·17

Method (4) was to method (5) as 1 : 1·68

Though the numbers are small and the testing clearly ought to be done on a larger scale, nevertheless the results select (4) and (5) as the best methods, and of these (5) is slightly the better.

Method (4), however, has the great advantage of simplicity, and does not require depressors, springs and dishes, which increase the chances of contamination and need frequent sterilization.

I, therefore, think it well to describe the fourth method in detail. Particles of fæces are scooped up from five different parts of the stool with bazaar made scoops. This fæces is placed in a test tube and shaken up with equal parts of methylated ether and hydrochloric acid (33 per cent), the emulsion strained through fine muslin and centrifuged. The fat and debris are carried or 'levitated' to the top, the ova with heavier debris are deposited below in a fine emulsion. This emulsion is sucked up by a pipette and as much as possible placed on the upper surface of a

coverslip resting on a slide, three coverslips being made by cutting an ordinary slide into three equal parts. Five minutes or more are allowed for settlement. The excess fluid is then pipetted off and the coverslip examined. If the emulsion is too thick for vision, it may be diluted and mixed with more of the ether and hydrochloric acid mixture, and, after a further five minutes for settlement, the excess again pipetted off.

Such is the method of searching for ankylostoma ova which seems to me the most efficacious at the present time.



# ESTIMATION OF ERYTHROCYTE AND HÆMOGLOBIN CONTENT OF BLOOD.

BY

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THE Thoma-Zeiss counting chamber is almost universally used for the purpose of estimating the number of erythrocytes in a given volume of blood. Under service and field conditions however, or where very large numbers of individuals have to be examined, there are certain disadvantages of this mode of examination. Apart from the possibility that the instrument itself may not be available, the chief disadvantage of its use, when a large number of cases have to be examined, is the time consumed, and the eye strain involved. There are also certain difficulties in the use of the instrument connected with the accurate filling of the bulb pipette and also with the satisfactory preparation of the specimen for examination which are sometimes vexatious. I have found the following methods easy to carry out, but have not been able to test them to any extent in cases of disease.

## I. ESTIMATION OF VOLUME OF ERYTHROCYTES.

The estimation of the volume of erythrocytes is the principle involved in the use of the hæmatocrit. There is no special reason why volume should not be as good a character by which to gauge the degree of anæmia as number. It has been customary, however, to use the numerical content instead of the volumetric content. Many objections have been urged against the use of volume as a measure. Thus it is said that the volume of erythrocytes varies greatly according to the type of anæmia. The volume of the individual corpuscle may be greatly increased in pernicious anæmias and not at all in secondary anæmias. If this objection is really valid and a

higher volumetric content were obtained in the former case than in the latter for an equal numerical content, then the volumetric method would be valuable as a means of differentiating the two types of anæmia. On the other hand, the erythrocytes in pernicious anæmia are probably more plastic than those in non-pernicious anæmias and so might be expected to pack away, by settlement, more completely than in the latter case. I think, however, that it is permissible, in the absence of proof of the seriousness of the objection, to doubt this statement, even although it seems to have the authority of consistent repetition in most text-books on the subject. Another objection is that it takes no account of the disturbing element of leucocyte content. But this does not seem to be a very serious objection. In the settlement of erythrocytes a large proportion, at all events, of the leucocytes come up to the top and are not included in the estimate of erythrocyte volume. In fact, this top layer of leucocytes could itself be measured in those diseases in which an increase takes place and thus the method might be used diagnostically in these diseases. Is it possible that these objections to taking volume of erythrocytes as a measure of anæmia are due more to the difficulties of using the hæmatocrit than to the reality of the objections themselves? I do not use any complicated type of tube for this estimation. Simple capillary tubes such as are used for the despatch of vaccine lymph serve the purpose, or the graduated capillary stem of a capillary pipette. The method is itself merely a variation of that described by Sir Almroth Wright many years ago. The chief points to be insisted on in the method are :—

- (1) The necessity for settlement to constant volume.
- (2) That the comparison throughout, until standards are so fixed as to render it unnecessary, be with a known normal blood under the same conditions of test.

#### *Method.*

(a) *Whole blood*<sup>1</sup>.—(1) Take a capillary tube of about 8 cm. length. (2) Make a mark<sup>2</sup> on the stem to leave roughly twice the length below the mark as above. (3) Measure with a steel measure the height of the mark from the lower end of the capillary, using a hand lens to read the measurement. (4) Fill in the capillary tube with blood<sup>3</sup> from a puncture to the mark. (5) Tilt the capillary tube to bring the blood away from the opening. (6) Pass the lower end straight into, and through, a small ball of plasticine, to close it. (7) Strengthen the plasticine closure by applying melted sealing-wax to the extremity and around it. (8) Close



the upper extremity with plasticine alone.<sup>4</sup> (9) Centrifuge the filled capillary tubes in the bucket of a hand centrifuge. (10) Examine them from time to time to determine whether settlement has reached constant level.<sup>5</sup> (11) Take the tubes out when this is attained and measure the height of the supernatant fluid.<sup>6</sup> (12) Calculate<sup>7</sup> the height of the column of erythrocytes. (13) Calculate<sup>8</sup> the volume of erythrocytes as a percentage on the total volume of blood used. (14) Calculate<sup>9</sup> the degree of deviation from normal.

*Notes.*—<sup>1</sup>The difficulties in using whole blood are that the manipulations have to be rapidly carried through in order to obtain settlement before clotting and that the centrifuging of erythrocytes in whole blood is much more difficult than in diluted blood. <sup>2</sup>The marking of the stem is very satisfactorily done by means of 'Chin Chin' or other type of waterproof Indian ink. A hypodermic needle serves as a marking pen. The *upper* level of the mark made is that taken in all measurements. It is well to mark at the same time the upper end of the capillary so as to distinguish it from the lower end. If 1, 2, 3, 4, and so on marks are made at this upper end they serve to differentiate the tubes from each other and these marks then enable them to be recognized as belonging to a particular case. <sup>3</sup>This can be done very accurately, for the blood flows up with greater or less rapidity according to the angle at which the latter is held. A little cedar wood oil may be filled into the lower extremity of the capillary before filling with blood. The oil is pushed up by the blood, and in its passage oils the inner wall of the capillary tube. This oiling serves to retard the rate of coagulation of the blood and it is easy to differentiate, after centrifuging, the three layers of fluid—oil at the top, then plasma, and the lowest the column of blood corpuscles. <sup>4</sup>The capillary is thus sealed completely and evaporation prevented. The question of evaporation applies more strictly to citrated blood. When closed in this way the capillary tubes may in the case of citrated blood be set aside to be treated at leisure. <sup>5</sup>The capillary tubes may be taken out and a pencil mark placed at the top of the column of erythrocytes. If the subsequent centrifuging shows that the erythrocytes have drawn away from this mark, then constancy of level has not yet been attained and the centrifuging has to be continued. <sup>6</sup>The supernatant fluid is measured and not the column of erythrocytes because in the sealing operations the lower end of the capillary has become blocked to a greater or less extent. <sup>7</sup>The difference between the height of the supernatant fluid and the original height of the column of whole blood will give the

height of the column of erythrocytes. If there is a leucocyte layer it will be included in the measurement of supernatant fluid. <sup>8</sup>This is done on the assumption that the capillary tube is of reasonably the same calibre throughout or does not differ to any extent above and below sufficiently to cause a significant error in the calculation. The calculation is:—

$$\frac{\text{Height of column of erythrocytes} \times 100}{\text{Height of column of whole blood}} = \text{percentage volume of}$$

erythrocytes. <sup>9</sup>In this method a sample of blood of a normal person is treated at the same time and in the same manner as that of the test case. Suppose such a sample of normal blood gave a volume of erythrocytes of 50 per cent and the test one of 45 per cent, then the test would show deficiency represented by the fraction  $\frac{15}{50} = 0.9$ .

(b) *Citrated blood.*<sup>1</sup> Make a mark<sup>2</sup> on the stem of a capillary pipette which is fitted with a teat. (2) Aspirate from a puncture a column of blood to reach the mark. (3) Eject the blood carefully into a watch-glass. (4) Aspirate an equal quantity of citrated salt solution<sup>3</sup> into the same pipette. (5) Eject into the watch-glass containing the blood. (6) Mix the blood and citrate by drawing up into, and rejecting from, the pipette. (7) Fill a marked and measured capillary glass tube with the required volume of the mixture, taking great care that settlement has not been allowed to take place in the watch-glass. (8) Close, and centrifuge in precisely the same way as in the case of whole blood. (9) Calculate in similar fashion the relation of volume of test blood to normal.

*Notes.*—<sup>1</sup>The advantages of citrated blood are obvious from the description of the disadvantages of whole blood. They are:—(1) that the work of centrifuging down to constant level is less hard; (2) that there is no need for any special hurry to get the centrifuging done, such as is necessary with whole blood, if it is not to clot. <sup>2</sup>It is sometimes advisable to make several marks, for the reason that it is not always certain that one will be able to get sufficient blood to reach to the mark made. It is useful then to have a choice of marks. The longer the column of blood taken the more accurate is the measurement. <sup>3</sup>1.5 per cent sodium citrate in 0.85 per cent salt solution. The blood is in this way diluted 2-fold and this will make the result differ from that of whole blood. This is of no consequence, as the method is one of comparison, under similar conditions, of test blood with normal blood. The volume

of erythrocytes obtained in the case of a normal citrated blood need not necessarily be exactly one half that of a normal whole blood estimation. That would depend on the isotonicity of the salt citrate solution with the blood plasma.

(c) *Citrated blood. Settlement by gravity*<sup>1</sup>.—(1) Use a piece of capillary tubing<sup>2</sup> or capillary pipette which has been graduated in equal volumes.<sup>3</sup> (2) Aspirate a given number of volumes of citrated blood into the pipette. (3) Withdraw the column of fluid slightly from the extremity. (4) Seal<sup>4</sup> the extremity. (5) Set upright in a test tube. (6) Read<sup>5</sup> the volume of the supernatant fluid after 24 hours. (7) Calculate the volume of erythrocytes. (8) Compare the volumes of test blood and normal blood for estimation of degree of normality.

*Notes.*—<sup>1</sup>This might be useful in the absence of a centrifuge although not so accurate. It is best in such a case to make a number of determinations as there is sometimes a liability to sticking of the column of corpuscles in the process of settling. The comparison should be made in each case with normal tubes as the attainment of constant level is very slow and would occupy many days. <sup>2</sup>If a piece of capillary tubing be used it may be graduated and afterwards filled by the device described under 'calibration with mercury.' <sup>3</sup>The unit volume may be quite arbitrary or a definite one such as 10 c.mm. It is convenient to make the number of volumes 10 for ease of subsequent calculation. <sup>4</sup>In the flame, by plasticine, or with sealing-wax. <sup>5</sup>It is necessary to compute by eye fractions of unit volume.

#### CALIBRATION OF PIPETTES BY MEANS OF MERCURY.

The first essential for this purpose is to have clean mercury. If the mercury be clean, a droplet can be picked up in its entirety by means of a pipette fitted with a teat. But if the mercury be not clean it becomes almost impossible to do so. Mercury may be cleaned by transference from one watch-glass to another, using a capillary pipette to effect the transference. The portion of mercury remaining, which as successive portions are withdrawn comes to show a scum, is rejected. Another method is to press the mercury through a clean linen handkerchief. The calibration of pipettes may be made with an arbitrary volume of mercury or with a known volume. The arbitrary volume method of calibration presents no difficulty. For a known volume it is necessary to have a standard volume pipette which must be carefully preserved

and kept clean. Such pipettes are conveniently of 10 c.mm. or 20 c.mm. volume.

(a) *Method of calibration with a definite volume.*

(i) *Capillary pipette.*—(1) Take a standard pipette and fit it with a teat.<sup>1</sup> (2) Pick up with careful exactness the volume of mercury required. (3) Test the exactness of the volume by bringing the mercury down to the extremity of the pipette and examining with a hand lens both the lower and the upper limit of volume. (4) Weigh the volume of mercury<sup>2</sup> obtained. (5) Pick up the mercury with a teated capillary pipette so that it occupies a position in the pipette from the extreme tip upwards. (6) Mark the upper<sup>3</sup> limit of the mercury with Indian ink by means of a hypodermic needle used as a pen and held in the left hand. (7) Aspirate the mercury up<sup>4</sup> the tube until its lower limit is exactly in contact with the already marked upper limit for unit volume. (8) Continue the process until 10 volumes or other number have been marked out.

*Notes.*—<sup>1</sup>The teat may require to be tied or wired on, or the pipette have its diameter increased at the upper extremity by the application of sealing-wax. <sup>2</sup>This, simply as a check on the accuracy of the volumetric measurement. The weight of this carefully taken volume should be established by previous trials. It should also be compared with the calculated weight of a given volume of metallic mercury. <sup>3</sup>The upper limit of the Indian ink mark should coincide exactly with the upper limit of the mercury. <sup>4</sup>By releasing gradually the pressure on the compressed teat. <sup>5</sup>If the pipette is to be a 10-volume pipette it is advisable to calibrate to 11 volumes. This allows for the withdrawal of the column of blood from the extremity for sealing purposes and renders computations of fractions of a volume easier.

(ii) *Capillary tube.*<sup>1</sup>—(1) Fit a piece of ordinary glass tubing with a teat at either end. (2) Bore a hole through the extremity of one of these teats with a fine glass capillary which has been heated for the purpose in the Bunsen flame. (3) Fit<sup>2</sup> the capillary tube into the hole thus made. (4) Use this instrument in the same way as a capillary pipette furnished with a teat. (5) Aspirate the given volume of mercury into the capillary. (6) Mark the position of the upper limit and continue the process of calibration to give 5 or other number<sup>3</sup> of volumes.

*Notes.* <sup>1</sup>*i.e.* where only the capillary portion of a pipette is used without any wide tube portion. <sup>2</sup>It should require some slight force to place in position. <sup>3</sup>If the volume calibration required be 5 it is better to mark in 6 volumes for the same reason as given above.

*(b) Method of calibration with an arbitrary volume.*

This is of precisely the same sort as the above, only that there is no necessity in this case to have a standard volume measuring pipette.

## DEGREE OF ACCURACY OF RESULTS.

Criticisms of the above methods on the score of accuracy may be adduced. Thus, (1) the capillary stem of a pipette or piece of capillary tubing is not of the same calibre throughout. The length occupied by a given volume of erythrocytes or supernatant fluid, as the case may be, will differ in different parts. It is to be remembered, however, that the comparison in this method is always with a 'normal' either at the time or by previous trials, and the 'normal' is subject to the same error of computation as the test. (2) The speed and duration of revolution of the centrifuge will affect the degree of settlement of the erythrocytes. Here again it is to be remembered that we are dealing only with relative values and the 'normal' is subject to the same conditions as the 'test.' By continuing to centrifuge until the erythrocytes come to occupy *constant* volume the necessity of using a 'normal' control each time becomes less insistent. It would be possible no doubt to have manufactured capillary tubing of exactly the same bore throughout a limited length, but this would mean some limitation of the ease of application of the test with ordinary laboratory materials. The following are some of the results obtained by the method showing the degree of variation obtainable on one and the same sample of blood.

1. Whole blood filled into capillary tubing and centrifuged to constant volume.

No.	Height of whole blood column. cm.	Height of supernatant fluid. cm.	Height of column of erythrocytes. cm.	Percentage volume erythrocytes.
<i>(a) Human blood. (W. F. H.)</i>				
1	3.7	1.8	1.9	51.4
2	7.8	3.45	4.35	55.1
<i>(b) Sheep blood.</i>				
1	4.65	2.2	2.45	55
2	4.70	2.2	2.50	53

2. Blood (W. F. H.) mixed with an equal volume of citrated salt solution filled into capillary tubing and centrifuged to constant volume.

Sample.	Height of whole blood column. cm.	Height of supernatant fluid. cm.	Height of column of erythrocytes. cm.	Percentage volume erythrocytes.
1	5.51	4.14	1.37	24.9
2	5.99	4.60	1.39	23.0
3	5.80	4.52	1.28	22.1
4	5.89	4.50	1.39	23.6
5	5.75	4.40	1.35	23.5
6	6.19	4.80	1.39	22.5

The mean of these observations is 23.3 and the maximum deviations from the mean are 1.2 (5.2 per cent) and 1.6 (6.9 per cent). I may take as an example of a typical count in a Thoma-Zeiss chamber that given by Gulland and Goodall (1912) for purposes of comparison. The example is :—

In the first set of 16 squares	..	..	108
„ „ second „ „ „ „	..	..	96
„ „ third „ „ „ „	..	..	98
„ „ fourth „ „ „ „	..	..	120
„ „ fifth „ „ „ „	..	..	121

The mean value in this example is 108.6 and the maximum deviations from the mean are 12.6 (11.6 per cent) and 12.4 (11.4 per cent). These are higher deviations than are given by the figures obtained from volume by the method of centrifuging capillary tubes.

3. Blood of sheep mixed with an equal volume of citrated salt solution filled into capillary tubing and centrifuged to constant volume.

Sample.	Height of whole blood column. cm.	Height of supernatant fluid. cm.	Height of column of erythrocytes. cm.	Percentage volume erythrocytes.
1	6.6	4.85	1.75	26.5
2	6.2	4.65	1.55	25.0
3	6.4	4.80	1.60	25.0
4	6.17	4.60	1.57	25.3

4. Blood of sheep mixed with an equal volume of citrated salt solution and filled into volumetric capillary pipettes. The dilutions were made with citrate plasma so as to make certain that all the mixtures were of the same 'tonicity.' The settlement was gravitational and the quantity of each dilution used was 10 volumes.

Dilution of citrated blood (10 volumes).	Percentage content of citrated blood.	VOLUME OCCUPIED BY ERYTHROCYTES AFTER SETTLE- MENT FOR DEFINITE PERIODS.				
		24 hours.	48 hours.	96 hours.		144 hours.
				Observed.	Calculated.	
10-10 ..	100	7.1	5.8	4.0	..	3.5
9-10 ..	90	4.3	3.2	2.9	2.9	2.8
8-10 ..	80	4.4	3.2	2.5	2.6	2.3
7-10 ..	70	4.3	2.9	2.3	2.3	2.1
6-10 ..	60	4.4	2.6	2.0	1.9	1.9
5-10 ..	50	2.5	1.9	1.7	1.6	1.5
4-10 ..	40	2.3	1.6	1.3	1.3	1.0
3-10 ..	30	1.6	1.0	0.9	1.0	0.8
2-10 ..	20	1.5	0.5	0.5	0.6	0.5
1-10 ..	10	0.6	0.3	0.3	0.3	0.25

The example shows that the gravity settlement method may have unsatisfactory features. It would seem that some want of settlement (sticking ?) had taken place during the first 24 or 48 hours, and especially in the 10-10 dilution. After 96 hours the gradation, except for the 10-10 value, is much more what it should be. It may be that if the gravity method is used a much greater dilution of the blood than is represented by equal parts of blood and citrate salt solution should be employed. A calculation of theoretic values has been made for the 96 hours' result starting with dilution 9-10.

5. Estimation of degree of differentiation given by the method of centrifuging to constant volume. This was not fully worked out and the results are few.

Dilution of citrated blood.	No. of volumes used.	VOLUME OCCUPIED BY ERYTHROCYTES AFTER CENTRIFUGING TO CONSTANT VOLUME.	
		Observed.	Calculated.
20-20 .. ..	5	1.60	1.60
19-20 .. ..	5	1.50	1.52
18-20 .. ..	5	1.50	1.44
17-20 .. ..	5	1.30	1.36



## II. ESTIMATION OF BLOOD CONTENT BY TINT AND TURBIDITY.

If it were possible to obtain a fluid which would preserve blood without hæmolysis and which would preserve its colour likewise, there would be no difficulty in devising an instrument to show the variation of normal and pathological blood with regard to the combination of characters, colour and turbidity. A comparison of a given dilution of test blood with a series of dilutions of a standard blood would then afford an immediate indication of its grade with respect to the normal. These characters, tint and turbidity, represent the degree of concentration of erythrocytes in unit of volume. In the majority of cases the fact that the small number of leucocytes relative to erythrocytes is ignored would not signify. I have succeeded in obtaining a fluid which preserves the blood indefinitely from hæmolysis, but not one which preserves the tint of the blood. Formalin, 10 per cent in 0·85 per cent sterile salt solution, preserves the erythrocytes intact—actually fixes them—but unfortunately there is a steady change of tint of blood in the mixture, which goes on for several days, and which renders comparisons of tint with freshly taken blood impossible. If a reagent could be combined with the formalin which preserved or changed instantaneously and permanently the tint of the admixed blood without causing hæmolysis, then the object sought would be attained, but this I have not yet succeeded in doing. The only reagent which I tried was hydrochloric acid and it did not prove satisfactory. The method of estimation of concentration of erythrocytes by turbidity may, however, have a usefulness which is independent of tint and I therefore describe it as a method which can serve an occasional purpose.

*Method.*—(1) Make a stock standard suspension<sup>1</sup> consisting of 1 part blood of a normal person and 99 parts of 10 per cent formalin in 0·85 per cent sterile salt solution. (2) Have prepared a series of 15 tubes made out of tubing of the same calibre, about 8 cm.  $\times$  0·4 cm. (3) Fill into tube No. 1 a quantity of stock standard suspension. (4) Make a series of suspensions, 9 parts stock to 1 part formalin salt solution, 8 parts stock to 2 parts formalin salt solution and so on down to 1 part stock to 9 parts formalin salt solution. (5) Fill in a quantum from each suspension into a separate tube. (6) Seal off the tubes in a blowpipe flame. (7) Mark them 10, 9, 8, . . . . . 1, with a writing diamond. (8) Use the remaining tubes as testing tubes. (9) Make a 1 per cent suspension

of patient's blood in 10 per cent formalin salt solution. (10) Fill in a quantity of this suspension into one of the testing tubes. (11) Compare<sup>2</sup> the opacity of the suspension so made with that in the series of standard tubes. (12) Calculate<sup>3</sup> the concentration of the erythrocytes in the patient's blood from the result.

*Notes.*—<sup>1</sup>This may be done in two stages by making first a 1-10 suspension and then diluting this again 1-10. Keep this suspension in a test tube, which can be easily shaken up, and so the corpuscles quickly brought again into suspension when required. <sup>2</sup>By holding the two tubes, standard and testing tubes, over well lighted print and making comparison of the visibility of the print through the testing-tube with that through a succession of standard tubes until they show equal.<sup>3</sup> The calculation is given as 10-10, 9-10, 8-10, etc. of normal according as the testing tube with its suspension corresponds to standard tube 10, 9, 8, etc.

The difficulty of obtaining a measure of tint and turbidity by means of a standard series of tubes, due to steady change of colour from the fresh state, was got over by employing an entirely different technique for measurement. The instrument used was the tinturometer, described by me in the October number of this journal (1919). The principle of this instrument is the use of a graduated cylinder in which a given dilution of the blood under test is contained. The reading consists in determining the residue in cubic centimetres of blood dilution left in the cylinder when the point is reached, at which, by continued subtraction of the fluid, standard visibility of definite sized print or of a figure such as that on a steel scale is attained. The cylinder for this reading is placed over the print or number and the reading taken by looking vertically downward through the fluid which it contains. By using this method one is not dependent on a fixed scale of colour or grade of turbidity against which to match the test object but upon a simple, single point of visibility. Here it is the instrument value which—other things being equal—requires to be fixed for the normal, or which, failing such fixity, is determined afresh on each occasion. Large numbers of estimations can be done at one time and at leisure; for all that is required at the time of examination is to obtain blood, including normal blood, from the subjects and dilute to a given degree. These dilutions may then be taken to the laboratory for the necessary measurement. The following table shows the results obtained with this instrument in the case of 6 apparently healthy men. The blood was diluted first 1-100 with

1·5 per cent citrated normal salt solution and a further 10-fold dilution of this made in the tinturometer itself.

Initials of individual.		Reading of the tinturo- meter.	
W.F.H.	..	..	0·8
K.R.K.I.	..	..	0·8
B.R.	..	..	0·9
S.L.	..	..	0·9
H.	..	..	1·0
B.	..	..	0·9

### III. ESTIMATION OF HÆMOGLOBIN.

The principle here used is that of Sahli's hæmoglobinometer, with the alteration that the comparison is made with a descending series of successive dilutions of erythrocytes derived from a normal individual. A stock standard 1 per cent solution is prepared, which is made by liberating the hæmoglobin from 1 volume of blood with 9 volumes of N-10 hydrochloric acid, then diluting this 10-fold with N-10 hydrochloric acid. A number of tubes, 15 in all, is made from the same piece of glass tubing and 10 of these are filled in with dilutions representing 10-10, 9-10, 8-10, . . . . . 1-10 of the stock standard solution. The five empty tubes are testing tubes and the test is carried through in precisely the same way as with the tubes similarly constructed for the turbidity test. The fluid in the tubes takes up immediately, under the action of the hydrochloric acid, a permanent tint, and so the difficulties experienced with the measurement of the turbidity of the blood do not interfere with the application of the method in this case. With keeping, however, the tubes show a deposit at the bottom due in all probability to the stroma of the erythrocytes taking up colour again from the solution. The fact does not interfere with the use of the tubes as all evidence of want of solution disappears on shaking up the tubes.

It is not absolutely necessary to compare a series of standard tubes with the test fluid. It suffices to proceed on the Sahli principle and use, say, a No. 5 or No. 3 tube only of the series given above. One would then take and dilute the test blood 1-100 and place one unit volume in a testing tube. Compare this in tint with the No. 5 or No. 3 standard tube and add unit volumes of diluent in succession until the tint of the mixture tallies exactly with that of the standard tube. A simple

calculation then gives the degree of excess or deficiency over normal of the test blood.

The tinturometer can also be very satisfactorily used as a hæmoglobinometer. The measure cylinder is first standardized by means of normal blood laked with N-10 hydrochloric acid. This may have to be repeated on each occasion of test, unless with repetition it is found that the result is sufficiently constant to allow of this being dispensed with. The test blood is laked and diluted in the same way as the normal blood and the measurement is carried out in the same fashion. Here also the measurements can be carried out in the laboratory at one's leisure, after the withdrawal of the blood has been effected and the blood appropriately diluted.

The following table shows the results obtained with the tinturometer in the case of 10 apparently healthy men. The blood was diluted at 1-100 with N-10 hydrochloric acid. The readings refer to this dilution.

Initials of individual.		Reading of the tinturometer.	
W.F.H.	..	..	1.4
S.L.	..	..	1.4
A.R.	..	..	1.3
E.W.M.	..	..	1.4
G.M.	..	..	1.5
Ch.	..	..	1.4
R.J.	..	..	1.4
K.	..	..	1.5
A.G.	..	..	1.5
A.H.	..	..	1.4

## ON THE USE OF BIRDS AS LABORATORY ANIMALS.

BY

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THE common animals in use in bacteriological laboratories are rabbits and guinea-pigs. In a country like India and still more in a country like Mesopotamia these are neither easily procurable nor cheap. It is a great desideratum in such localities to be able to procure small animals which can take the place of these well-tried servants of the laboratory. The animal which immediately suggested itself for trial was the common fowl. Its utilization for the production of high titre agglutinating serum was suggested by the work of Lt.-Col. W. D. Sutherland, I.M.S., Imperial Serologist, on the very important precipitating sera used in forensic medicine. The trial has been quite successful, and it may be said that in countries where fowls abound—and where do they not?—the smallest of laboratories need not be at a loss for the production of their own high titre agglutinating sera. Attempts were made to determine whether fowls would furnish hæmolytic sera, such as could be used in Wassermann tests. The trials which I have so far made have failed; the serum produced in response to intravenous inoculation of sheep's erythrocytes showed only a greatly increased agglutinating power for corpuscles and very little hæmolytic power. Various references are made in bacteriological literature to the pathogenicity of certain disease-producing organisms for fowls and pigeons, and doubtless these could, to some extent at least, serve as laboratory animals for the purposes of such tests. The convenience of these animals in regard to ease of procural, ease of housing, and ease of handling, and their cheapness will not be questioned. I have used, in the production of high titre agglutinating sera, guinea-fowls, fowls, and pigeons and

the number of trials of these various birds is now considerable. Fowls have a moderate sized wing vein and give a good serum. Guinea-fowls have a large wing vein, but their blood occasionally shows defective and secondary coagulations which interfere to some extent with the yield of serum. I have used the blood of guinea-fowls aspirated into definite quantities of citrated salt solution for the procural of an agglutinating plasma, which if further diluted gives satisfactory results as an agglutinating serum. For its size the pigeon is even more satisfactory than fowl or guinea-fowl. The amount of serum obtainable from it is, of course, small in comparison with the other two birds, but it has a comparatively large wing vein for purposes of inoculation and withdrawal of blood, and the sera given by it are of distinctly good titre.

*Technique of production of sera.*—(1) Use in all the birds the series of doses of living bacteria equivalent to weights of 1, 2, 3, and 4 mgm. of dried bacterial substance, or possibly a slightly lesser dosage, seeing that this series gives rise to loss of weight. (2) Administer these doses intravenously at intervals of one week. (3) Test the potency of the serum ten days after the last injection. (4) Use a large bore 20 c.c. syringe needle for bleeding, if the serum is up to titre. (5) Pass the needle into the wing vein, which should be denuded of feathers, and collect the blood,<sup>1</sup> which issues in drops, in a test tube. (6) Place the test-tube immediately in the sloped position in the incubator at 37°C. for 2 hours. (7) Remove the test-tube and set vertical. (8) Leave overnight and pipette off the serum which has collected in the morning. (9) Test the potency of the serum. (10) Dilute with 0.85 per cent sterile salt solution containing 0.5 per cent carbolic acid, to give an agglutinating titre of 1 in 1000 for the specific organism. (11) Issue for use.

*Notes.*—<sup>1</sup>Quantities such as 5 c.c. and 10. c.c. of blood from one wing vein are easily obtainable from the pigeon and fowl and larger quantities from the guinea-fowl. The inoculations may be continued after withdrawal of blood and the bleeding repeated on subsequent occasions.

#### *Examples of method.*

I. *Fowl No. 6.*—Given doses intravenously at weekly intervals of living *B. typhosus*, equivalent to weights of 1, 2, 3 and 4 mgm. of dried bacterial substance. Weight of fowl for these intervals 750, 730, 680 and 650 gm. Titre 1 in 8000, without the end point being reached. The 'titre' is given by the highest dilution of serum in which flocculation of the bacterial suspension is evident to the naked eye.

II. *Pigeon No. 19.*—Given doses intravenously at weekly intervals of living *B. typhosus*, equivalent to weights of 1, 2, 3 and 4 mgm. of dried bacterial substance. Weight of pigeon for these intervals 300, 260, 250 and 230 gm. Titre 1 in 8000.

III. *Guinea-fowl No. 34.*—Given doses intravenously at weekly intervals of living *B. paratyphosus A.* equivalent to weights of 1, 2, 3 and 4 mgm. of dried bacterial substance. Weight of guinea-fowl for these intervals 870, 850, 820 and 800 gm. Titre 1 in 1000.

#### *Conclusions.*

1. Birds can satisfactorily take the place of the rabbit in laboratory practice for the production of high titre agglutinating sera.

2. Birds have not proved useful for the production of anti-sheep hæmolytic sera.



# A BACTERIOLOGICAL INVESTIGATION OF INFLUENZA.

CARRIED OUT UNDER THE INDIAN RESEARCH FUND  
ASSOCIATION

BY

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## PART I.

*Influenza in Calcutta, February to May 1919.*

THIS report deals with a bacteriological study of influenza and its complications carried out at Calcutta in February, March, April and May 1919, during the period of comparative quiescence which followed the epidemic of November and December 1918.

### NATURE OF CASES.

The patients from whom clinical material was obtained belonged to the poorer and less intelligent classes of the community. The majority of them sought admission to hospital apparently as a last resort, having been ill at home for several days up to two weeks. Many arrived in a moribund condition, 47 per cent of the total number of deaths occurring before the patients had been four days in hospital. Practically all of them were suffering from bronchitis or broncho-pneumonia, and none could be considered as early cases of influenza.

Their ideas with regard to the onset and course of the disease were hazy and unreliable, nor could absolute reliance be placed on their description of symptoms. All cases diagnosed as broncho-pneumonia, acute bronchitis, lobar pneumonia or influenza have been included here under the term 'influenza.' Lobar pneumonia was rarely found.

Spinal puncture was performed on a number of patients with symptoms of meningitis, but as it was not a routine procedure, and as epidemic cerebro-spinal fever was present in Calcutta at the time, a few cases of so-called 'influenza with meningeal symptoms' may have been due to the meningococcus. It is also possible that one or two cases of cholera may have been included under the heading 'influenza with gastro-intestinal symptoms.'

#### CLINICAL PICTURE.

A detailed description of the clinical picture presented by these cases is beyond the scope of this report. The writer used to visit the hospitals two or three times a week in order to 'make rounds' with the physicians-in-charge and see new cases. The notes recorded below represent the general impression received by one who was a frequent visitor to the wards, and are not the result of an analysis of case reports.

Signs of respiratory affection were manifest in every case, though varying in kind and degree. Bronchitis was nearly always present and broncho-pneumonia common; tubular breathing and massive consolidation were rarely encountered. Dyspnoea and cyanosis were prominent symptoms and quite marked even when physical signs of pneumonia were not prominent. There were few patients without cough. In the feeble and prostrated the cough was usually unaccompanied with expectoration; in others, the sputum was yellowish or greenish, purulent, sometimes foetid and often expectorated in large quantity but seldom rusty. Hæmoptysis was of rare occurrence.

Frontal headache was a very common symptom. Some patients complained of dull aching occipital pain, others of earache. There were only a few cases of otitis media; this may, however, be a late complication and a more frequent one than hospital experience indicates. Insomnia was a striking feature in many cases.

An interesting case was that of a man with a chronic lachrymal fistula of two years' standing, who was admitted for influenza. On the day after admission the fistula, which had been dry, began to discharge pus, the bacteriological examination of which gave a pure culture of pneumococcus and Pfeiffer's bacillus; other organisms appeared at a later date.

This patient subsequently developed a fatal pneumococcal meningitis.

On walking through the wards one was struck by the abnormal mental condition of many of the patients. Some were drowsy and apathetic, others restless or even delirious, and occasionally one would see an unconscious patient or one with definite signs of meningitis: neither paralysis of the cranial nerves nor hemiplegia was noted.

Diarrhœa, with passage of bloody stools, was sometimes met with, but this condition was not persistent and seemed to yield readily to treatment.

The temperature charts usually showed irregular remittent fever falling slowly by lysis. Some patients had high continuous fever resembling that of acute pulmonary phthisis. Others (and some of these were severe cases) were afebrile, or nearly so, throughout their illness.

The most striking features of influenza as seen in these cases were:—

- (1) The prostration ('low condition') and toxic appearance of the patients even in the absence of marked physical signs or high temperature.
- (2) Dyspnoea out of all proportion to the amount of lung tissue affected.
- (3) The abnormal mental condition of the patients.
- (4) The high mortality.

#### BACTERIOLOGICAL EXAMINATION.

Sputum, naso-pharyngeal secretion, blood, pleural and cerebrospinal fluids were examined in order to determine what organisms were most prevalent in the body fluids and secretions of influenza patients.

#### SPUTUM.

##### *Collection of Material.*

Samples of sputum were collected in sterile wide-mouthed bottles or large test tubes. No preparation of the patient's mouth was made beforehand; he was merely instructed to cough and spit into the sterile receptacle. A few patients were too prostrated to cough up material from the lungs or bronchi; from these a certain amount of naso-pharyngeal secretion mixed with saliva was taken for examination.

##### *General Characters.*

Careful records were kept of the gross appearance of 88 samples of sputum collected from patients with broncho-pneumonia during the

second week of the disease. An analysis of the descriptions given shows that the character of the sputum varied considerably in different patients. 89 per cent (79) were characterized as 'muco-purulent,' 11 per cent (9) as 'mucoïd and watery.' In 74 per cent (65) the expectoration was considered to be 'copious,' while in 26 per cent (23) it was 'scanty.' Of the muco-purulent sputa 18 per cent (14) were 'blood-stained,' 8 per cent (6) had a fœtid odour, and 62 per cent (49) were greenish in colour.

Although no type of sputum can be considered as characteristic of influenza at this stage of the disease, it is worthy of note that no less than 55 per cent of all sputa examined were described in the following terms:—

'Copious expectoration: sputum contains masses of greenish purulent material mixed with thin watery mucoïd fluid.'

There also seems to be a relation between this kind of sputum and the presence of Pfeiffer's bacillus, for the bacillus was isolated from 26 out of 34 specimens of greenish purulent sputum (76 per cent), while it was isolated 41 times from 68 sputa of all descriptions (60 per cent) collected during the same period and sown on the same kind of culture media. Further, the pus collected from the accessory respiratory sinuses, *post-mortem*, in which Pfeiffer's bacillus predominated, was most frequently of this peculiar colour.

#### *Microscopic appearance.*

Smears were made from a large number of specimens of sputum and stained by Gram's method. I found that this gave some information with regard to the number and variety of organisms present, and the quantity of sputum to be sown so as to give discrete colonies on a blood agar plate. It was not possible, however, to diagnose Pfeiffer's bacillus with any great certainty in the stained smears, except when the bacilli appeared in characteristic clusters in the large endothelial or polynuclear cells.

In some cases many organisms resembling Pfeiffer's bacillus were seen in the stained specimen, yet culture failed to give positive results. In others, positive cultures were obtained, when microscopic examination seemed to be negative, and this most frequently when there was no evidence of phagocytosis: small Gram-negative organisms lying free on a reddish background are apt to be overlooked. It is possible too that 'involution forms' occur in sputum—they undoubtedly occur

in pus from the accessory respiratory sinuses—and this would tend to make a direct diagnosis largely a matter of guess-work.

In my opinion Pfeiffer's bacillus cannot be recognised with any degree of certainty in stained smears of sputum.

Staphylococci and the 'catarrhalis group' present little difficulty; often, however, there is confusion between pneumococci and non-hæmolytic streptococci, unless, and sometimes even when, a capsule stain is employed. No attempt was made by cultural methods to differentiate between the meningococcus and the various members of the 'catarrhalis group.'

#### *Method of sowing.*

Small fragments of muco-purulent material, pellets of sputum or threads of mucus were picked out, washed several times and finally suspended by trituration in a few cubic centimetres of salt solution. A loopful, or part of a loopful, of the suspension was smeared over a blood-agar plate and incubated at 37° C. for 48 hours. In the early part of the investigation two plates were used for each specimen. Later on (when the use of heated pigeon's blood was introduced) only one plate was employed.

In several instances a portion of the sputum was injected into the peritoneal cavity of a mouse.

#### *Culture media employed.*

At the beginning of the inquiry, specimens of sputum were sown on 3 per cent nutrient agar (Baird and Tatlock's peptone) containing 4 per cent human blood, the agar being +3 to +5 (Eyre's scale). It was thought that by using this medium alone the work would be simplified and at the same time the more delicate organisms inhabiting the respiratory tract would be readily isolated. Subsequent work proved this idea to be erroneous.

Fifty-six sputa were examined by this method (see Table 1). Fifteen of these were plated on human blood agar enriched with hydrocele fluid. In no case did the enriching fluid appear to encourage the growth of Pfeiffer's bacillus.

In the subsequent work two other media were employed.

- (1) Nutrient agar containing 2·5 per cent *unheated* pigeon's blood.

(2) Nutrient agar containing 1 per cent *heated* pigeon's blood.

Each specimen was sown on two plates, the first containing 4 per cent human blood agar and the second one of the media mentioned above.

The following tables give a result of the examination of a large number of samples of sputum, each sample being taken from a different patient, and present evidence of the value of so-called '*selective-media*.'

TABLE I.

*Results of the examination of 56 specimens of sputum planted on 4 per cent human blood (unheated) agar.*

Organism.		Numerous colonies. Per cent.	Few colonies. Per cent.	TOTAL.	
				Positive. Per cent.	Negative. Per cent.
Pneumococcus	..	66	27	93	7
Streptococcus	..	34	52	86	14
Pfeiffer's bacillus	..	16	5	21	79

TABLE II.

*Results of the examination of 46 specimens of sputum on 4 per cent human blood (unheated) agar and 2.5 per cent pigeon's blood (unheated) agar.*

Organism.	HUMAN BLOOD AGAR.		TOTAL.		PIGEON'S BLOOD AGAR.		TOTAL.	
	Numerous colonies.	Few colonies.	Positive.	Negative.	Numerous colonies.	Few colonies.	Positive.	Negative.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
<i>Pneumococcus</i>	76	22	98	2	66	28	94	6
<i>Streptococcus</i>	31	58	89	11	26	54	80	20
<i>Pfeiffer's bacillus</i>	18	16	34	66	34	18	52	48

TABLE III.

*Results of the examination of 22 specimens of sputum on 4 per cent human blood (unheated) agar and 1 per cent pigeon's blood (heated) agar.*

Organism.	HUMAN BLOOD AGAR.		TOTAL.		HEATED PIGEON'S BLOOD AGAR.		TOTAL.	
	Numerous colonies.	Few colonies.	Positive.	Negative.	Numerous colonies.	Few colonies.	Positive.	Negative.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
<i>Pneumococcus</i>	74	26	94	6	62	24	86	14
<i>Streptococcus</i>	28	56	84	16	24	36	60	40
<i>Pfeiffer's bacillus</i>	17	22	39	61	46	32	78	22



This series brought to light some interesting facts.

(a) *Pfeiffer's bacillus.*

(1) Pfeiffer's bacillus grows far better on pigeon's blood, heated or unheated, than on human blood (unheated): the colonies are larger, less transparent and more easily detected.

(2) On human blood agar it is necessary to allow incubation to continue for 48 hours or even 72 hours; the colonies of Pfeiffer's bacillus are definitely larger at 48 than at 24 hours and they are more easily seen. This is due in part to their greater size and opacity and in part to the fact that other organisms may have altered the medium, rendering it transparent in places.

On pigeon's blood agar, however, the colonies after 24 hours' incubation are usually larger and more opaque than those which have been growing on human blood agar for 48 hours, and are thus more readily detected.

(3) Transparent media (*i.e.*, media containing heated blood) are preferable to opaque media (*i.e.*, media containing unheated blood). On the latter, colonies of Pfeiffer's bacillus often pass unobserved on account of their minute size and the fact that they are almost invisible when viewed by transmitted light and only appear clearly when the plate is held at certain angles, and viewed by reflected light. By the use of transparent media these difficulties are overcome.

(4) Inexperienced workers have little difficulty in picking out colonies of Pfeiffer's bacillus on heated pigeon's blood agar. Their appearance is quite characteristic:—round, convex, with a distinct nipple-like projection in the centre, transparent, of a faint yellow colour and margin entire. Further, when one is dealing with many sputa every day, there is a great saving of time and media: 1 per cent blood gives excellent results; one plate is sufficient for each sample of sputum and the colonies can be readily recognised and 'fished' after 18 to 24 hours' incubation.

(5) There are two organisms which I have encountered, whose colonies on heated pigeon's blood agar resemble those of Pfeiffer's bacillus: a member of the 'catarrhalis group' and a diphtheroid bacillus. The colonies of the former are very transparent but not umbonated (nipple-centred), those of the latter are translucent and umbonated, but more opaque than Pfeiffer's bacillus.

*(b) Pneumococcus and Streptococcus.*

(1) Pigeon's blood seems to have some inhibitory action on the growth of pneumococci and streptococci, and the viability of these organisms is less than on human blood agar.

(2) The colonies of pneumococcus are smaller on pigeon's blood (heated or unheated) than on human blood, flat, with a double contoured, often raised, margin and resembling to some extent a rope quoit. This appearance is usually evident after 48 hours' incubation.

(3) The pneumococcus colony on heated pigeon's blood agar is characteristic, but this is not so in the case of the streptococcus. The colonies of the latter are very small; no pigment is produced, and it is almost impossible to differentiate between hæmolytic and non-hæmolytic varieties.

*Staphylococcus albus* and *aureus* and members of the 'catarrhalis group' grow well on this medium.

*Summary.*

(1) A transparent solid medium containing heated blood is more satisfactory for the isolation and identification of Pfeiffer's bacillus than ordinary blood agar.

(2) The use of such a medium is most essential where large numbers of sputa have to be examined, and where time and laboratory staff are limited.

(3) Heated pigeon's blood has given the best results in my experience. The results of the experiments in which different kinds of blood were used are not included in this report, but, as will be shown later, heated human or rabbit's blood may be used satisfactorily.

(4) Ordinary blood agar (human, rabbit or sheep) should be used for isolating pneumococci and streptococci.

*Method of obtaining blood from a pigeon's heart.*

In a pigeon the sternum is roughly boat-shaped; the 'keel' and 'gunwhale' are composed of very dense bone while the intervening portions are made of cancellous bone, which varies in thickness and density in different areas. An oval patch of membrane about  $\frac{1}{2}$  inch long by  $\frac{3}{8}$  inch wide occupies an area on each side of the sternum, the middle of which corresponds to a point about  $\frac{3}{4}$  inch below the manubrium and  $\frac{3}{4}$  inch laterally.

Pluck off the breast feathers of a pigeon: sterilize the skin with alcohol; insert the needle through the skin at a point 1 inch below and 1 inch to the side of the manubrium, and push it through the muscle slightly inward and forward so as to strike the membranous portion of the sternum. A little pressure will now send the needle through the membrane into the heart, and blood can be withdrawn by means of the attached syringe.

The needle may be inserted through the membrane on either side of the sternum, as may be found convenient: and the operation may be carried out without any assistance if the pigeon be held in one hand with its head tucked into the pocket of an ordinary laboratory over-all worn by the operator and the syringe employed be one that is easily manipulated with the other hand (*e.g.*, a Roux 20 cc. syringe).

Five cubic centimetres of blood may be withdrawn from a pigeon every three or four days for a considerable period (I have had some pigeons in constant use for over four months), and as much as ten cubic centimetres on a single occasion.

The method is simple; no assistance is required; the bird is easily handled and remains quiet during the operation. After one has become moderately expert the mortality is very slight, no greater indeed than in guinea-pigs as the result of a similar operation.

#### *Preparation of heated pigeon's blood agar.*

Five cubic centimetres of blood are added to 45 cubic centimetres of 1 per cent sodium citrate in normal salt solution; the mixture is heated at 66° to 70° C. for half an hour and filtered through filter paper.<sup>(1)</sup> One cubic centimetre of the filtrate is added to every 10 cubic centimetres of nutrient agar at a temperature of 50° to 60° C. The medium is of a faint yellow colour and transparent.

#### IDENTIFICATION OF THE VARIOUS ORGANISMS.

##### *(1) Pfeiffer's bacillus.*

Smear preparations are made from a number of likely looking colonies and stained by Gram's method. Suspicious colonies containing Gram-negative bacilli are then sub-cultured on blood agar slants—at first 4 per cent human or rabbit's blood agar, later on 1 per cent heated

pigeon's blood agar was the medium used for subcultures. If the agar slants are dry, several colonies can be separately subcultured on one and the same slant. After 18 to 24 hours' incubation at 37° C., smears are made from each growth and stained by Gram's method. Hanging drop preparations are then made from growths of small Gram-negative bacilli, and those that are non-motile are subcultured on blood agar and on plain agar. If after 24 hours' incubation growth has occurred on blood agar but not on plain agar, the organism is considered to be Pfeiffer's bacillus.

The diagnosis, based as it is on morphological characters which vary with the age of the culture and the medium on which it is grown, on a single staining reaction, on non-motility and the absence of spores, and finally on the hæmophilic property of this organism, is necessarily uncertain.

I was, however, able in a number of instances to test certain other properties of Pfeiffer's bacillus and thus render the diagnosis more exact.

These experiments, it is hoped, will form the subject of another communication. It is sufficient to say that the great majority of the strains isolated from sputum, lungs and accessory respiratory sinuses produced *indole* when grown on heated blood agar, and that while growing on 1 per cent pigeon, human, rabbit and sheep's blood with varying degree of luxuriance, they nearly always failed to grow in the presence of 1 per cent goat's blood.

None of the strains grew on plain agar or on plain agar containing hydrocele fluid.

## (2) *Pneumococcus*.

The isolation of pneumococcus did not present great difficulty. The characteristic appearance of the colonies, the green discolouration of the medium, and the morphological and staining characteristics were sufficient to differentiate it from all other organisms save non-hæmolytic streptococci.

The final tests were those of bile solubility and fermentation of inulin. The bile test proved to be very reliable; at least all of the strains which fermented inulin as well as some of the non-inulin fermenters were bile soluble.

Staining for capsules was not a routine procedure, and relatively few strains were passed through mice. Agglutination tests were carried out with a certain number of strains.

(3) *Streptococcus*.

After isolating hæmolytic and non-hæmolytic colonies, the various strains were planted on lactose, mannite and salicin broth containing Andrade's indicator. All of the strains fermented lactose, a certain number produced acid in salicin broth, but none fermented mannite. Green pigmentation of the colonies and surrounding medium was a very common finding.

The differentiation of hæmolytic from non-hæmolytic streptococci by examining blood-agar plates with the naked eye or hand lens is not always an easy matter. If, however, the colonies be examined with a 3rd objective or by the method described below, it will be seen that some of them produce *complete* hæmolysis in the surrounding medium both at the side of and underneath the colony, and that none of these strains develop pigment. Others, pigmented as well as non-pigmented, show surrounding zones of partial hæmolysis varying in width and clarity, and clusters of unaltered red cells immediately underneath the colonies.

Since the hæmolysis produced by different strains varies in degree, it is likely that individual workers have different ideas of what are 'hæmolytic' and 'non-hæmolytic' colonies.

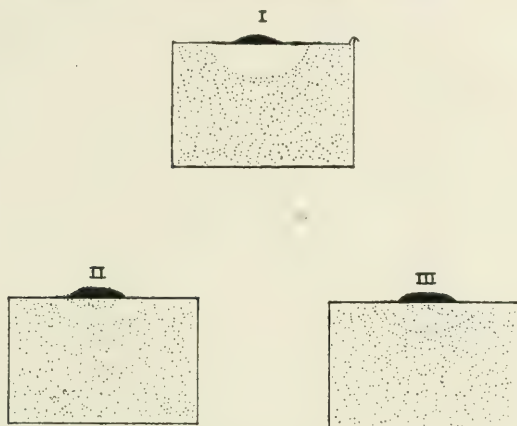
In this paper only those colonies that produced *complete* hæmolysis of the surrounding medium have been termed 'hæmolytic.'

In the series of sputa examined, all of the hæmolytic strains belonged to the *pyogenes* group. All the non-hæmolytic strains belonged to the *salicarius* and *mitis* groups<sup>(2)</sup>, that is, the groups most commonly found in the normal respiratory tract.

*Method of differentiating hæmolytic from non-hæmolytic streptococci<sup>(3)</sup>.*

Punch out with a cork borer a cylinder of agar, including a colony and a portion of the surrounding medium: with a sharp safety-razor blade make a thin vertical section through the colony and the entire thickness of the underlying agar. Lay the section flat on a clean glass slide and examine with a hand lens and the low power of the microscope.

The following types of hæmolysis may be illustrated :—



I. Complete hæmolysis.

II and III. Partial hæmolysis.

TABLE IV.

*Result of the examination of the sputum of influenza patients. (The figures in brackets represent the total number of specimens examined.)*

Pneumococcus . . . . .	96% (124)
Streptococcus—	
(a) Viridans . . . . .	63%
(b) Non-hæmolytic non-pigmented . . . . .	16%
(c) Hæmolytic . . . . .	7%
* Pfeiffer's bacillus (heated pigeon's blood agar) . . . . .	78% (22)
Diphtheroids (heated pigeon's blood agar) . . . . .	32% (22)
Catarrhalis group . . . . .	28% (124)
Staphylococcus aureus . . . . .	13% (124)

#### BLOOD.

Fifty-six blood cultures were taken during life. The blood was planted in glucose broth, and plain agar (roll cultures and poured plates);

\* See Tables I, II and III.

no anaerobic methods were employed. *Pneumococcus* was the only organism isolated.

Number of blood cultures . . . . .	56
<i>Pneumococcus</i> only . . . . .	14
No organisms . . . . .	39
Contaminated . . . . .	3

Ten out of the 14 positive cultures were obtained within 48 hours of the death of the patient.

#### CEREBRO-SPINAL FLUID.

Number of specimens examined . . . . .	22
<i>Diplococcus intracellularis</i> only . . . . .	7
<i>Pneumococcus</i> only . . . . .	1
No organisms . . . . .	12
Contaminated . . . . .	2

Of these cases 18 were tentatively diagnosed influenza with meningeal symptoms, and four epidemic cerebro-spinal meningitis, pending the bacteriological report on the cerebro-spinal fluid.

The seven cases in which the meningococcus was found will be left out of account. In every one of the remaining fifteen cases the cerebro-spinal fluid came out under pressure. It was clear or very slightly turbid in 14 cases, and contained a few pus cells but no organisms; in the remaining case there was a heavy deposit of pus together with pneumococci. All of the specimens gave a strongly positive reaction for globulin when tested by Nogouchi's butyric acid method.

The specimens were centrifuged, the fluid poured into 50 c.c. flasks containing glucose blood broth, and such sediment as could be obtained was smeared over the surface of a pigeon's blood agar plate. Smears were stained by Gram's method.

No organisms were found either in smears or on culture, except in the case of pneumococcal meningitis. Blood cultures from two of the patients revealed a pneumococcal septicæmia. One of these was the patient with purulent cerebro-spinal fluid mentioned above.

#### POST-MORTEM EXAMINATIONS.

The post-mortem examinations were carried out under the great disadvantage that as a rule the bodies could not be opened until 24 hours after death, and cold storage was not available. On this account



complete autopsies were not made, the first two attempts proving such procedure to be futile from a bacteriological point of view.

Examination of the accessory respiratory sinuses, however, gave information of value, their inaccessibility rendering them comparatively free from saprophytic organisms even when the soft tissues had begun to decompose<sup>(4)</sup>.

Fourteen autopsies were done and the brain, accessory sinuses and middle ears examined in each case.

Oedema of the brain was common, and the cerebral vessels were usually distended. The subarachnoid space and the ventricles often contained a large amount of fluid which was only slightly turbid and contained few cells. Only one case showed definite meningitis with a plastic exudate.

In nine cases pus was found in one or more of the sinuses. The quantity of pus varied to a great extent: in some instances it was only detected by scraping a platinum loop along the lining membrane of the sinus, in others several cubic centimetres were collected. In two cases the sphenoidal sinuses contained so much pus that on cutting into the lining membrane a veritable purée of greenish-yellow stuff poured out over the base of the skull. There was a marked variation, too, in the quantity of pus found in the corresponding sinuses of opposite sides as in the size and shape of the cavities; in one case the left frontal sinus was rudimentary. The sinuses or middle ear of one side sometimes contained a considerable amount of pus while those of the other side were apparently normal. In two cases pus was found in the mastoid cells, but there was no evidence of bone necrosis. In no instance could any extension of inflammation be traced from the sinuses to the dura mater.

*Bacteriological examination of pus from the accessory respiratory sinuses.*

The bacteriological findings are open to criticism, on account of the time elapsing between the death of the patient and the autopsy. The organisms seen in smears or isolated by culture were Pfeiffer's bacillus pneumococcus, staphylococcus, albus and aureus, streptococcus, diplococcus catarrhalis, Gram-positive bacilli large and small, and long slender Gram-negative bacilli. Pfeiffer's bacillus or pneumococcus was always the predominating organism. They are considered to be the chief

pathogenic agents in the sinusitis of these cases for the following reasons :—

- (1) Pfeiffer's bacillus or pneumococcus was found in every sinus save one, when pus was present.
- (2) They were always present in overwhelming numbers, other organisms in small numbers.
- (3) They were always phagocytosed by leucocytes, the others rarely so.
- (4) Staphylococci were found in two cases only.
- (5) The Gram-negative and positive bacilli which were isolated proved to be gas producers and were almost certainly saprophytes.
- (6) In two autopsies which were done within eight hours of the death of the patient, *B. influenzae* and pneumococcus were recovered in pure culture.

Stained smears of pus invariably showed Pfeiffer's bacillus in far greater numbers than pneumococcus. In all cases Pfeiffer's bacillus and pneumococcus were afterwards isolated in pure culture on pigeon's blood agar. The following gives a resumé of the results of the nine post-mortem examinations in which there was evidence of sinusitis.

TABLE V.

Case No.	FRONTAL SINUSES.		ETHMOIDAL SINUSES.		SPHENOIDAL SINUSES.		MIDDLE EARS.		CEREBRO-SPINAL FLUID.		REMARKS.
	Pus.	Pfeiffer's bacillus and Pneumococcus.	Pus.	Pfeiffer's bacillus and Pneumococcus.	Pus.	Pfeiffer's bacillus and Pneumococcus.	Pus.	Pfeiffer's bacillus and Pneumococcus.	Pus.	Pfeiffer's bacillus and Pneumococcus.	
M. 13	+	+	—	—	—	—	—	—	—	—	* Pneumococcus but no B. Pfeiffer.
M. 26	+	+	+	+	+	+	+	+	—	—	
M. 29	+	+	+	+	+	+	+	+	—	—	
C. 133	+	+	+	+	+	+	+	+	—	—	
C. 142	—	—	+	+	+	+	+	+	+	+	Pus in mastoid cells.
C. 156	—	—	—	—	+	+	+	+	+	+	
C. 167	+	+	+	+	+	+	—	—	—	—	Large flakes of pus at base of brain chiefly in interpeduncular space. Purulent fluid in ventricles. Pus in mastoid cells.
C. 206	+	+	+	+	+	+	—	—	—	—	† B. Pfeiffer but no pneumococci.
C. 210	+	+	+	+	+	+	—	—	+	—	† B. Pfeiffer but no pneumococci.
							+	present.			
							—	absent.			

*Heart and Lung punctures.*

In a number of cases material for culture was obtained by puncturing the heart and lungs with a needle of large bore as soon as possible after the death of the patient.

The results of the bacteriological examination of this material are given below :

Organisms.				Heart.	Lung.
Pneumococcus	..	..	..	25 (pure culture.)	2 (almost pure culture.)
Streptococcus (hemolytic)	..	..	..	2 (pure culture.)	8 (mixed culture.)
Mixed cultures containing pneumococcus and non-hemolytic streptococcus, but no Pfeiffer's bacillus.				12	23
Pfeiffer's bacillus	..	..	..	0	2 (almost pure culture.)
Mixed cultures containing Pfeiffer's bacillus, pneumococcus and non-hemolytic streptococcus.				0	16
Negative	..	..	..	20	0
TOTAL				59	51

Pigeon's blood agar was employed as the culture medium.

## AGGLUTINATION TESTS.

*Presence of agglutinins for Pfeiffer's bacillus in patients' sera.*

The sera of 39 patients were tested against the homologous strains of Pfeiffer's bacillus isolated from their own sputum. Thirty-two of these gave positive agglutination tests, the remaining seven were negative.

The agglutination titre was never high at any time, but definitely higher in the fourth week of the disease than in the first.

TABLE VI.

*Agglutination titre of the serum of 28 patients taken at different stages of the disease.*

1ST WEEK.		2ND WEEK.		3RD WEEK.		4TH WEEK.	
Case No.	Agglutination Titre.	Case No.	Agglutination Titre.	Case No.	Agglutination Titre.	Case No.	Agglutination Titre.
189	1 : 32	D. 16	1 : 32	J. 21	1 : 32	J. 22	1 : 32
240	1 : 32	D. 21	1 : 32	D. 25	1 : 32	J. 8	1 : 64
251	1 : 32	D. 26	1 : 32	J. 9	1 : 64	J. 12	1 : 128
272	1 : 32	187	1 : 32	J. 10	1 : 64	D. 31	1 : 128
238	1 : 64	213	1 : 32	J. 5	1 : 128	J. 13	1 : 256
		259	1 : 32	J. 11	1 : 128		
		D. 24	1 : 64				
		191	1 : 64				
		197	1 : 64				
		257	1 : 64				
		263	1 : 64				
		266	1 : 64				

The figures represent the highest dilution of serum with which complete agglutination occurred after two hours in a water-bath at 55° C. The bacterial suspensions were made from 24-hour growths on pigeon's blood agar in distilled water. Normal salt solution was used for diluting the sera. The readings were taken  $\frac{1}{2}$  hour after removing the tubes from the water-bath.

Four cases were chosen from among those in which the date of the onset of the illness was fairly definitely known, and a series of agglutination tests was done at six days intervals. The result of this experiment shows that agglutinins for the homologous strain of Pfeiffer's bacillus appear during the first week of the disease, increase during the next two weeks, reach a maximum between the third and fourth week and remain at this maximum point for at least a week longer.

TABLE VII.

	Case I.		Case II.		Case III.		Case IV.	
	A.	B.	A.	B.	A.	B.	A.	B.
A.—Day of disease .	7	1/16	6	1/32	7	1/16	8	1/32
B.—Agglutination titre.	13	1/32	12	1/32	13	1/32	14	1/64
	19	1/32	18	1/64	19	1/32	20	1/64
	25	1/64	24	1/128	25	1/64	26	1/128
	31	1/64					32	1/128
	37	1/64						

Samples of sputum were collected from these cases at the same time as the blood for the agglutination tests, and planted on pigeon's blood agar plates, and an attempt was made to count the colonies of Pfeiffer's bacillus on each occasion, in order to determine whether there was any relation between the number of organisms present in the sputum and the agglutination titre.

Very irregular colony counts were obtained, and this is not to be wondered at when one considers the difficulty in obtaining a representative sample of sputum, the small amount of the suspension actually sown on the plate, and the technical difficulties associated with sowing and with the recognition and counting of the colonies.

Agglutination tests were done with the sera of 52 patients against *heterologous* strains. The majority of the sera were obtained from patients during the second and third weeks of the disease. Four strains of Pfeiffer's bacillus were used for making the bacterial suspension, two from the lungs and two from the sphenoidal sinuses of fatal cases.

Twenty-two cases gave positive results, the titre of the serum varying from 1 : 8 to 1 : 64 ; thirty cases were negative.

Twenty-eight sera were put up simultaneously with homologous and heterologous bacterial suspensions. Twenty-three gave positive results with the homologous suspension, and of these only ten were positive with the heterologous suspension. None of the sera which were negative when put up with the homologous suspension gave a positive test with the heterologous suspension.

It is worthy of note that the agglutination titre of a serum was invariably lower when tested against the heterologous than against the homologous suspension, and that in the former test 'paradoxical agglutination' often occurred, *e.g.*, negative results might be obtained in the stronger concentrations of serum, 1 : 2 and 1 : 4, and positive results in 1 : 8, 1 : 16 and 1 : 32.

#### *Time of disappearance of agglutinins.*

It has been shown that agglutinins for Pfeiffer's bacillus appear in the blood of influenza patients during the first week of the disease, and persist there for at least one month longer, and that in about 40 per cent of the cases, agglutination of heterologous strains occurs.

An attempt was therefore made to determine the time of the disappearance of these antibodies from the blood. It was impossible to

carry out this experiment with the sera of hospital patients and homologous strains, as the patients were rarely kept in hospital for longer than five weeks.

There had been, however, an epidemic of influenza at the Presidency and Central Jails, during the latter part of 1918, and more than 100 of the inmates, who had been attacked with influenza at that time, were still in prison in May 1919.

Blood from 27 men who had had influenza in July, October, November, or December 1918 was collected in May 1919, and the various sera tested against the same heterologous strains as were previously used against patients' sera. Not one of the sera gave a positive reaction in dilutions ranging from 1 : 2 to 1 : 64.

If it be assumed—(1) that these men were infected with Pfeiffer's bacillus during their attack of influenza, and (2) that the infecting strains possessed serological properties (agglutinability or antigenic power) in common with the test strains, the conclusion may be drawn that agglutinins for Pfeiffer's bacillus disappear from the blood during the first six months after an attack of influenza.

#### *Control Tests.*

The blood of healthy individuals and of patients suffering from tuberculosis, lobar pneumonia, Bright's disease, Hodgkin's disease, ankylostomiasis, kala azar, and certain surgical conditions, *e.g.*, hernia and hydrocele, were tested with the heterologous suspensions mentioned above.

In all, the sera from six healthy and 19 diseased persons were tested. One case of lobar pneumonia with empyema and one case of ankylostomiasis, without any history of influenza, gave a positive reaction, the others were negative.

#### *Conclusions.*

(1) The blood serum of influenza patients infected with Pfeiffer's bacillus possesses the power of agglutinating the homologous strain (82 per cent of cases).

(2) Agglutination of heterologous strains occurs in about 40 per cent of the cases.

(3) The serum of healthy persons does not agglutinate Pfeiffer's bacillus.



(4) Agglutinins appear in the blood of patients during the first week of the illness, and persist for at least five weeks afterwards.

*Types of pneumococcus found in influenza.*

Twenty-three strains of pneumococcus isolated from the sputum, blood, pleural fluid and accessory respiratory sinuses were tested with Mulford's high titre agglutinating sera, Types I, II, III.

Seventeen of these strains were non-agglutinable and were therefore considered to belong to Group IV (American classification).

The results of the agglutination tests of the six positive cases are given below :

Strain.	Origin.	Serum.	DILUTIONS.				1/64	1/128	Bile Solu- bility.	Inulin Fer- men- tation.
			1/4	1/8	1/16	1/32				
M. 29	Sphenoidal sinus <i>post-mortem.</i>	I	—	—	—	—	—	—	+	+
		II	+	+	+	—	—	—		
		III	—	—	—	—	—	—		
C. 41	Blood <i>ante-mortem</i>	I	+	+	+	+	—	—	+	—
		II	+	+	+	—	—	—		
		III	—	—	—	—	—	—		
C. 103	Blood <i>ante-mortem</i>	I	—	—	—	—	—	—	+	Weak.
		II	+	+	+	+	—	—		
		III	+	+	+	—	—	—		
C. 127	Blood <i>post-mortem</i>	I	—	—	—	—	—	—	+	Weak.
		II	+	+	+	+	+	+		
		III	+	+	+	+	+	—		
C. 135	Sphenoidal sinus <i>post-mortem.</i>	I	—	—	—	—	—	—	+	+
		II	+	+	+	—	—	—		
		III	+	—	—	—	—	—		
C. 190	Blood <i>ante-mortem</i>	I	+	+	+	+	—	—	+	+
		II	+	+	—	—	—	—		
		III	—	—	—	—	—	—		

Serum used—Mulford's Type I Serial number 877.

" " II " " 918.

" " III " " 908.

The finding that 72 per cent of the strains did not belong to either of the three 'fixed' types of pneumococcus is in agreement with the results of American workers, who point out that the pneumococci most commonly found in influenzal pneumonia are members of a heterogeneous group (Group IV).

The results of the positive tests differ so markedly from other published results that they must be accepted with reservation. They are recorded here because they are the findings of tests carefully made and repeated no less than nine times, using on some occasions 24 hours' broth cultures, on others a saline suspension of a 24 hours' growth on blood agar, and carried out at temperatures of 37° C. and 55° C., the readings being recorded after two hours' incubation and after 18 hours in the ice-box.

The age of the test sera was not known, the serial numbers on the bottles conveying no information in this respect, and so one cannot escape from the possibility that the results may have been vitiated by the use of sera which had lost their specificity by age or otherwise.

#### ANIMAL EXPERIMENTS.

Only a few animal experiments were carried out during this period of the investigation. A brief summary of some of the findings which are of interest are recorded here.

- (1) *Mouse inoculation as a method of recovering Pfeiffer's bacillus from the sputum of influenza patients.*

Fourteen white mice were inoculated intraperitoneally with sputum from the same number of influenza patients. All of them died within 24 hours. Pfeiffer's bacillus and pneumococcus were isolated from the heart's blood of 11, pneumococcus alone from 3; material from the peritoneal cavity also gave cultures of these organisms, mixed with staphylococcus, streptococcus, and a fairly large Gram-negative bacillus. Mouse inoculation would seem, therefore, to be a satisfactory method for recovering Pfeiffer's bacillus from sputum.

In the hot weather, however, a large number of experiments on these lines failed to give satisfactory results, as the majority of the mice dying during the night were found to be infected with a Gram-negative motile bacillus which spread so rapidly and extensively over the blood agar plates as to render the isolation of other organisms a most difficult matter.

The wild brown mouse is readily infected with pneumococcus and Pfeiffer's bacillus and may be used instead of the white mouse.

- (2) *Attempts to infect rabbits, guinea-pigs, pigeons and mice by intravenous and intraperitoneal inoculation of live cultures of Pfeiffer's bacillus.*

(a) The experiments with guinea-pigs and pigeons gave negative results.

(b) Forty-two rabbits were inoculated intravenously with live cultures (about 2,000 millions B. Pfeiffer). It was found that 4 hours and 8 hours after inoculation the organisms could be recovered from the heart's blood, 16 hours after inoculation, however, the blood was free from organisms. In 11 instances the animals died within 48 hours after inoculation, but no organisms were recovered from the blood, brain, spleen or liver. I have not been able to account fully for their death, but the post-mortem findings of an increase of fluid in all the serous cavities, dilatation of the right heart, and petechial hemorrhages on the pleuræ and pericardium suggest the possibility of toxæmia.

A Pasteurella infection was the cause of death in 26 rabbits which were inoculated with live cultures of Pfeiffer's bacillus. A note on the first six cases has been published in the *Indian Journal of Medical Research* (October 1919).

(c) *Mice.*

Eleven strains of Pfeiffer's bacillus were injected intraperitoneally into white and brown mice. With large doses (about 1,000 millions) the animals developed a fatal septicæmia within 48 hours. With small doses, e.g., about 10 millions, septicæmia was not invariably produced. It was found, however, that if a small dose of pneumococcus be injected together with Pfeiffer's bacillus, septicæmia always supervened, both organisms being recovered from the heart's blood.

This observation seems to me to be of considerable importance as it suggests the symbiotic growth of these two organisms *within the body*, with a possible increase of virulence in one or both.

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# THE PRODUCTION OF INDOLE BY PFEIFFER'S BACILLUS.

## A SIMPLE LABORATORY METHOD OF DETECTING THIS ORGANISM IN THE SECRETIONS OF THE RESPIRATORY TRACT.

BY

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INDOLE is one of the products of the decomposition of proteins which contain a tryptophane group, and certain bacteria during their growth and multiplication in culture media, containing free tryptophane or a tryptophane polypeptide group, have the power to effect this decomposition with the production of indole.

As this property is not common to all bacteria it has considerable value as a means of identifying and classifying bacterial species.

Early work on the production of indole by *B. coli* gave such variable results that doubts were entertained with regard to the usefulness of the indole test, but it was shown later that such irregularities were due in a large measure, to the technical methods employed.

Since 1905, when Böhme applied Ehrlich's delicate rosindole reaction to the study of bacteria, indole production has been more carefully investigated. Various sources of error have been eliminated and now the detection of minute traces of indole in bacterial cultures is an easy matter.

Only a small number of pathogenic bacteria are known to produce indole. In Kolle and Wassermann's *Handbuch für pathogenen mikroorganismen*, 1913, Pfeiffer's bacillus is not mentioned among them.

Zipfel (quoted by Jordan)<sup>(1)</sup> mentions four groups of indole producers:—'*B. coli* group, *B. dysenteriae* vars. Flexner and Y, cholera vibrios, and cholera-like vibrios except V. Finkler-Prior and V. Deneke. Pfeiffer's bacillus was apparently not among those tested.'

Early in this year Marcel Rhein<sup>(2)</sup> and Jordan<sup>(1)</sup> pointed out that certain strains of Pfeiffer's bacillus were able to produce indole when grown in suitable culture media, and the latter, after a series of experiments, came to the conclusion that a hæmophilic organism was the cause of indole formation in cultures taken from the throats of normal persons, and that this organism was presumably Pfeiffer's bacillus.

These observations, if correct, are of considerable importance, as they add to the certainty with which Pfeiffer's bacillus can be identified and further, suggest the possibility that the presence of this organism in sputum and naso-pharyngeal secretion may be detected by a simple chemical test, thus eliminating the elaborate and somewhat uncertain bacteriological technique at present employed.

As a result of investigations carried out since last August, I have been able to confirm these observations and have now accumulated sufficient evidence to warrant the conclusion that, with proper precautions, the indole test offers a means of recognising Pfeiffer's bacillus in sputum and naso-pharyngeal secretion which is more accurate and far easier of application than the usual methods practised in bacteriological laboratories.

#### *Technique employed.*

The material to be examined is planted on two slopes, one of plain agar, the other of heated pigeon's blood-agar, prepared according to the method devised by Dr. Soparkar<sup>(3)</sup>.

The cultures are incubated for 24 hours at 37° C. At the end of this time, the cotton wool plug of each tube is moistened with about six drops of Ehrlich's reagent, and two drops of a 5 per cent aqueous solution of potassium persulphate.\* The tubes are then placed in boiling water for about fifteen minutes. Indole being volatile is driven off, and as it comes in contact with the moist cotton wool the rosindole reaction is observed, the colour varying from rose to mauve.

Now, while the tubes are still warm, two cc. of Ehrlich's reagent and 0.5 cc. of the persulphate solution are floated on the surface of the melted agar. A positive test is indicated by a rose coloured ring at the junction of the agar and the reagents, which deepens to a cherry red on standing for a few minutes.

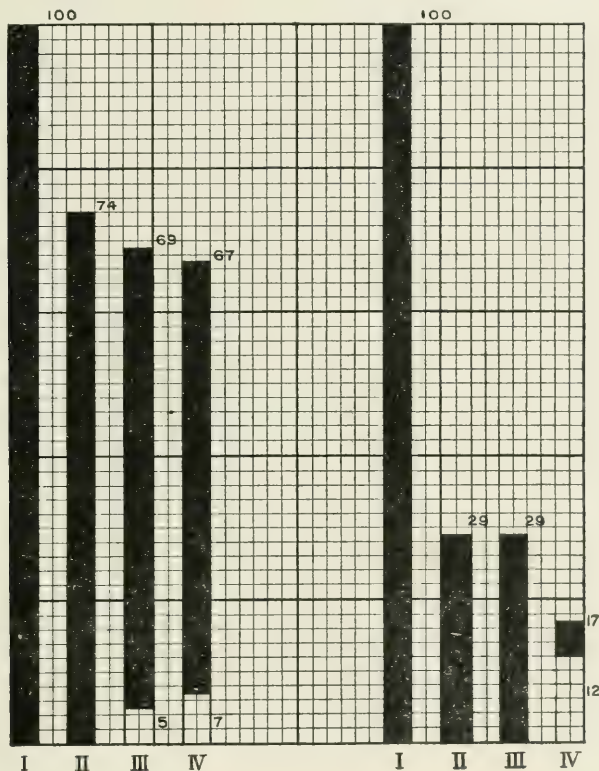
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\* According to McConkey the persulphate may be omitted; this has been fully borne out by the experience gained since writing this paper.

Chart showing the comparative value of the indole reaction method and the plate cultural method of detecting Pfeiffer's bacillus.

Influenza patients

Healthy persons



I - 100 specimens.

II - Total number of times Pfeiffer's bacillus was detected.

III - Number of times Pfeiffer's bacillus was detected by the indole reaction method.

IV - Number of times Pfeiffer's bacillus was detected by the plate cultural method.

The white columns represent the percentage of failure attributable to each method.

The depth of colour varies with different cultures, and depends, I believe, on the amount of indole present, provided that constant quantities of the reagents be employed.

The tubes are gently shaken and the red fluid is pipetted off into small test tubes containing 2 cc. of methylated chloroform. A violet ring forms at the junction of the two liquids, and on shaking and gently warming, the chloroform assumes a violet tint while the supernatant fluid gradually loses its red colour.

The method described here permits three variations of the test to be performed on one and the same culture. The cotton wool plug test, suggested by Dr. S. N. Goré of the Bombay Bacteriological Laboratory, serves to show that the substance giving the colour reaction is a volatile one. The chloroform extraction method controls the ring test and eliminates false indole reactions in which the cherry red colour is insoluble in chloroform.

Control tubes should also be tested to show that the culture medium itself does not contain indole nor give a false reaction. In this connection it must be noted that certain brands of commercial peptone are unsuitable. I have obtained satisfactory results with Baird and Tatlock's bacto-peptone, and with the casein digest used at the Bombay Bacteriological Laboratory.

#### *Results of the experiments.*

In all 182 specimens have been examined; 99 were sputa and *post-mortem* material from influenza patients and 83 throat swabs from healthy persons.

In every case two methods were used for detecting the presence of Pfeiffer's bacillus. (1) A portion of the material to be examined was sown on heated pigeon's blood-agar plates and incubated for 24 hours at 37° C. The colonies were then 'fished' and subsequently identified. (2) Another portion was sown on plain agar and blood-agar slopes and tested for indole in the manner just described.

The accompanying chart shows that the presence of Pfeiffer's bacillus was detected by both methods combined in 74 per cent of the specimens from influenza patients and in 29 per cent of the throat swabs from healthy persons. In 3 per cent of the influenza cases the indole method failed, while the plate method succeeded; in 7 per cent the plate method failed, while the indole method succeeded. Of the healthy



cases the plate method failed in 12 per cent, while no failures were recorded against the indole method.

The failures of the indole method were due to two sources of error. In three cases the strains of Pfeiffer's bacillus happened to be non-indole producers and so escaped detection, in the remaining two the result was vitiated by the presence of an indole-producing organism which grew on plain agar.

In the plate method the failures (approximately 19 per cent) were due, in part to the technical difficulties associated with plating, and in part to the non-recognition or absence of colonies of Pfeiffer's bacillus.

(It should be remembered that the plates must be thinly sown in order to obtain discrete colonies, while the test tubes may be heavily sown. Colonies of Pfeiffer's bacillus when they are of minute size or present in small number may be overlooked, while, on the other hand, even a few colonies on the agar slope will give rise to a demonstrable amount of indole. Indeed Ehrlich's reagent is delicate enough to detect the indole produced by a single *isolated* colony. It is also possible that heavy sowing may have the effect of enhancing the growth of Pfeiffer's bacillus through symbiosis with other organisms, and so give the indole reaction method a further advantage over the plate method.)

In four instances the growth on plain agar gave a positive indole reaction. Three of these were specimens of sputum from influenza patients with bronchiectasis, and the fourth was a sample of pus taken *post-mortem* from a case of broncho-pneumonia with empyema. In every instance the specimen had a distinctly foetid odour, and in two of them Pfeiffer's bacillus was isolated by the plate method.

#### *Interpretation of the results.*

In interpreting the results of these experiments the following inferences have been drawn :—

- (1) If a blood-agar tube be positive and the corresponding plain agar tube negative, the culture contains an indole producing strain of Pfeiffer's bacillus.
- (2) If both blood-agar and plain agar tubes be positive the culture may or may not contain an indole-producing strain of Pfeiffer's bacillus in addition to an indole-producing organism which grows on plain agar.
- (3) If a blood-agar tube be negative, and the corresponding plain agar tube positive or negative, the culture does not contain an indole-producing strain of Pfeiffer's bacillus.

In drawing these inferences the assumption is made that Pfeiffer's bacillus is the only haemophilic indole-producing organism to be found in the sputum and naso-pharyngeal secretion of healthy persons and of patients suffering from influenza. This is difficult to prove, but there is good reason to believe it to be true.

There is no evidence in the literature that any of the common organisms of the respiratory tract are indole-producers, and, as far as I am aware, no *haemophilic* organism, save Pfeiffer's bacillus, comes under this category.

Zipfel<sup>(1)</sup> tested, among others, a number of strains of *staphylococcus albus*, *aureus* and *citreus*, *streptococcus haemolyticus*, *B. tuberculosis*, *B. pneumoniae* (Fränkel), *B. diphtheria*, *B. mucosus ozaena*, *B. rhinocleromatis*, and *B. pestis*, and found them to be non-indole-producers.

Jordan<sup>(1)</sup> tested 36 strains of *streptococcus viridans*, *pneumococcus meningococcus* and *staphylococcus* with uniformly negative results, and I have confirmed this with other strains of the same species as well as with various diphtheroid organisms and members of the catarrhalis group.

Again, if the method is to be of value as a routine procedure, it must be shown that the non-indole producing strains are in the minority. This in my experience is the case. I have examined 104 strains of Pfeiffer's bacillus isolated from various sources and only 8 of these did not possess the indole-producing property.

Rhein<sup>(2)</sup> found 7 indole-producers out of 8 strains tested, and Jordan<sup>(1)</sup> 10 out of 13.

It seems probable, therefore, that the predominating varieties of Pfeiffer's bacillus are indole-formers.

It has been seen that there are two main sources of error in the method advocated.

(1) The presence of indole-producing organisms which are capable of growing on plain agar. This condition has appeared in 2 per cent of the specimens taken from influenza patients, but not in the throat swabs from healthy persons. The error is readily detected, and may be corrected by resorting to the ordinary cultural methods.

(2) The presence of non-indole-producing and the absence of indole-producing strains of Pfeiffer's bacillus in a given specimen.

In this case the error remains undiscovered, but has been practically negligible (less than 10 per cent) in my series of experiments with pure cultures and with mixed cultures. It must, however, be borne in mind

hat under different conditions, *e.g.*, in a non-epidemic period, or in different localities, the percentage of non-indole-producing strains may possibly be considerably greater than I have stated.

*Summary.*

(1) 92 per cent of the strains of Pfeiffer's bacillus tested produce indole when grown in suitable culture media.

(2) No other hæmophilic organism present in the respiratory tract of healthy persons or of influenza patients is known to possess this property.

(3) The indole reaction can be used as a means of identifying Pfeiffer's bacillus in pure cultures, and recognising its presence in mixed cultures.

¶(4) The method described here is claimed to be more accurate and more easily applied than the laboratory methods usually employed for the recognition of Pfeiffer's bacillus in the secretions of the respiratory tract of influenza patients.

It should also serve as a rapid and easy means of detecting 'carriers' in a healthy population.

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# LETHARGIC ENCEPHALITIS IN KARACHI DURING AN EPIDEMIC OF INFLUENZA.

BY

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INFLUENZA reappeared as an epidemic in Karachi in November 1919, the previous epidemic having occurred in August 1918. The present epidemic has been widespread, with a death rate which, in December, was nearly three times as great as in normal times.

It was not until the middle of November 1919 that lethargic encephalitis made its appearance. The first case diagnosed as such was that of a European male, aged 20 years, who had lately arrived from a ship on which cases of influenza had been reported—the patient himself was not known to have suffered from influenza. From the time he arrived in Karachi he was considered to be dull and stupid. On November 9th he was thought to be suffering from hysteria; on the 10th he was extremely drowsy, showed unilateral ptosis, but no facial paralysis. His speech was clear but languid, his hearing somewhat dulled; knee-jerks were increased but no pathological reflexes were present. On the 12th he remained flaccid in bed, unable to speak or to take solid food and motionless except for some twitching of the right side of the face. His neck muscles were stiff, Kernig's sign was present but knee jerks were

absent. There was slight fever. Next day the drowsiness was more marked; there was a rise in temperature, restlessness, insomnia and retention of urine. The temperature rose to 105 F. before death. The post-mortem record showed that there was no evidence of meningitis; 'hæmorrhagic patches on the surface of the brain were observed.' The cerebrospinal fluid taken during life was turbid.

Neither of us had seen this patient, and some doubt exists whether this was a case of lethargic encephalitis. The history and post-mortem findings suggest this possibility. Turbid cerebrospinal fluid, however, has not been observed in any of the cases subsequently occurring in Karachi.

From November 10th until December 27th, twenty cases have occurred, three at the British Station Hospital, one at the Indian Station Hospital and sixteen in the city. There have been seven deaths.

TABLE I.

*List of the cases of Lethargic Encephalitis occurring in Karachi City  
between November 10th and December 27th, 1919.*

No.	Caste.	Sex.	Age in years.	Date reported to the Health Officer.	Approximate date of onset of illness.	REMARKS.
1	Jewish ..	Male	14	..	15-11-19	Recovering.
2	Mohammedan	Male	40	21-11-19	..	Died, 21st November 1919.
3	Christian ..	Male	28	22-11-19	22-11-19	Died, 3rd December 1919.
4	Mohammedan	Male	30	23-11-19	23-11-19	Recovering.
5	Mohammedan	Male	26	23-11-19	23-11-19	Recovering.
6	Mohammedan	Male	30	..	23-11-19	Recovering.
7	Mohammedan	Female	50	25-11-19	..	Died, 25th November 1919.
8	Mohammedan	Female	12	3-12-19	3-12-19	Recovering.
9	Hindu ..	Male	10	3-12-19	3-12-19	Recovering.
10	Christian ..	Male	30	3-12-19	3-12-19	Recovering.
11	Christian ..	Male	35	3-12-19	..	Died, 7th December 1919.
12	Jewish ..	Male	65	7-12-19	..	Died, 9th December 1919.
13	Parsi ..	Female	19	7-12-19	..	Recovering.
14	Hindu ..	Female	20	7-12-19	..	Died, 12th December 1919.
15	Hindu ..	Male	28	9-12-19	19-11-19	..
16	Hindu ..	Male	20	27-12-19	16-12-19	..
17	Mohammedan	Male	26	..	30-11-19	Recovering.

(Indian Station Hospital.)

Three other cases have been reported at the British Station Hospital.

Through the kindness of the medical attendants we have been able to examine a number of these patients. The history, symptoms and course of the disease were so similar to those reported in Europe and America that the diagnosis was scarcely in doubt.

Case No. 1 had just recovered from an attack of jaundice when he suddenly developed diplopia, slight fever, headache, constipation and retention of urine. Though usually of a quiet disposition he now became restless and excitable, exhibiting choreiform movements. Following this lethargy appeared, and the patient remained in a somnolent condition for more than a week. During this period he required catheterization and enemata.

When seen by us, five weeks after the onset, the following signs were present:—double ptosis, dilatation, fixation and inequality of the pupils, paralysis of the left side of the face, marked tremor of the hands and a somewhat staggering gait. The squint had disappeared but he was still lethargic and languid in speech and movements. When last seen, January 5th, he was much improved mentally. Paralysis of the 3rd and 7th cranial nerves was still present, but not as marked as before.

The illness in Case No. 8 began suddenly with slight fever, headache and constipation; two days later lethargy appeared. She remained quiet in bed, not opening her eyes or speaking for about two weeks. She was seen by us for the first time three to four weeks after the onset. Her condition was one of extreme lethargy; when shaken and spoken to loudly she would answer questions rationally. She had double ptosis, pupils were dilated and equal, reacting to light sluggishly; no facial paralysis was noticed. Hands, feet and eyelids were somewhat œdematous. There was a bed-sore on the occipital region. Knee-jerks were not elicited; no pathological reflexes were present. A lumbar puncture was made, the patient moaning a little during the operation. Ten days later (January 2nd) she had definitely improved, and was energetic enough to brush a fly away from her face; the pupils, however, were still somewhat dilated. There was no facial paralysis; knee-jerks were now present.

The history of the onset in Case No. 9 was very similar to that of the preceding case. The illness began abruptly with fever, headache, vomiting, constipation and sleeplessness. For the first few days he was very restless and irritable, but soon became drowsy and apathetic. For days at a time his bowels were not moved even with comparatively large doses of calomel. When seen on December 26th, about four weeks

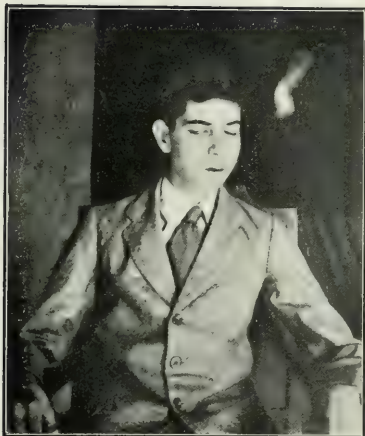


FIG. 1.

Case 1, 50 days after the onset of the illness. The patient is trying to close both eyes.



FIG. 2.

Showing the appearance of Case 8, 23 days after the onset of the illness.



FIG. 3.

Case 9, 23 days after the onset of the illness.



FIG. 4.

Case 9, 30 days after the onset of the illness







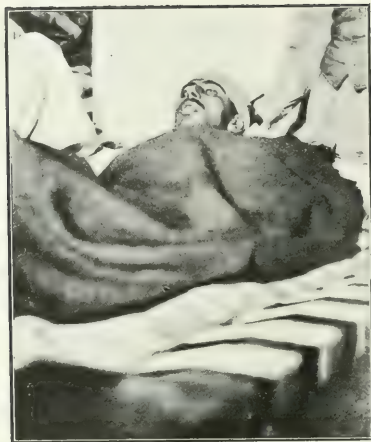


FIG. 5.

Case 16, 11 days after the onset of the illness.



FIG. 6

Case 17, 34 days after the onset of the illness.  
The patient has been asked to close both eyes.



FIG. 7.

Case 17, 34 days after the onset of the illness.  
Showing paralysis of the left side of the face.



FIG. 8.

Case 17, 'curing' himself by gazing into a concave  
mirror.

after the onset, the following signs were present :—double ptosis, nystagmus, pupils widely dilated, unequal and fixed, head somewhat retracted, knee-jerks increased ; Kernig's sign was present. He could not be induced to smile or speak, but was able to swallow liquids.

A lumbar puncture, with ethyl chloride as a local anæsthetic, failed to disturb his state of apathy. On December 28th no improvement was noticed. On January 2nd he smiled and in doing so paralysis of the left side of the face was revealed. His respirations were very hurried (36 per minute) but there was no evidence of pneumonia or pleurisy. He developed bed-sores on his back during the illness.

In Case No. 17 the onset was sudden with slight fever, headache and constipation. He was admitted to hospital in a semi-conscious condition. Two days afterwards, signs of 3rd and 7th nerve paralysis were observed. In this case, as in others, lethargy appeared after a few days of insomnia and restlessness.

Case No. 16 gave a history of fever for two days followed by headache, restlessness and sleeplessness and then by lethargy. He developed 3rd nerve paralysis and later on paralysis of the 9th nerve and incontinence of urine.

It is not our object to describe in detail the clinical aspect of this disease. The brief notes given above serve to show, however, that we have been dealing with the same condition that has been described elsewhere as lethargic encephalitis.

In these, and other cases, which came under our observation, the most striking features were :—

- (1) Sudden onset, with headache or vertigo and slight fever.
- (2) A short period of restlessness and insomnia, followed by a longer period of lethargy and somnolence.
- (3) The rapid appearance of cranial nerve affection, involving most frequently the 3rd and 7th nerves.
- (4) Furred tongue and persistent constipation.
- (5) Choreic movements and tremor of the hands and lips.
- (6) Absence of catarrhal symptoms, bronchitis or pneumonia.
- (7) Slight fever for one or more weeks following the onset.

#### LABORATORY EXAMINATION.

The blood, cerebrospinal fluid and urine of a certain number of patients were examined, and the results given in Tables II, III and IV.

TABLE II.

Table showing the result of the examination of the blood of *lethargic encephalitis* patients—Karachi.

Case No.	Date of examination.	Duration of the illness.	Total leucocyte count.	Neutrophile polymorphs per cent.	Eosinophile polymorphs per cent.	Large monocytes.	Small monocytes.	Wassermann test.	Widal test.	Parasites.	REMARKS.
3	29-11-19	7 days	10,800	82	0	5	13	—	Negative	Nd	Died, 3rd December 1919.
4	27-11-19	4 days	11,900	73	0	11	16	—	Negative	Nd	....
5	26-12-19	33 days	7,800	66	3	7	24	Negative	Negative	Nd	Syphilis (treated with novarsenobillon).
6	29-11-19	6 days	10,200	86	0	4	10	—	Negative	Nd	....
8	26-12-19	23 days	13,400	—	—	—	—	Negative	—	Nd	Lethargic.
"	2-1-20	30 days	13,200	71	5	6	18	—	—	Nd	Improving.
"	4-1-20	32 days	13,500	67	6	8	19	—	Negative	Nd	Much improved.
9	26-12-19	23 days	12,200	—	—	—	—	—	Negative	Nd	Very lethargic.
"	2-1-20	30 days	14,600	79	3	8	10	Negative	—	Nd	No improvement.
"	4-1-20	32 days	—	78	2	8	12	—	—	Nd	Slight improvement.
10	2-1-20	29 days	11,250	65	2	9	24	—	—	Nd	Almost completely recovered.
15	29-11-19	10 days	10,300	75	2	9	14	—	—	Nd	....
16	29-12-19	13 days	10,300	74	0	11	15	—	—	Nd	....
17	2-12-19	3 days	14,100	82	1	7	10	Negative	Negative	Nd	Very ill.
"	3-1-20	34 days	13,500	67	6	8	19	—	—	Nd	Almost recovered.

TABLE III.

Table showing the result of the examination of cerebrospinal fluid obtained from tetanic encephalitis patients -Karachi.

Case No.	Date of examination.	Duration of illness.	Appearance.	Pressure.	Cells.	Globulin test.	Reduction of Fehling's solution.	Wassermann test.
4	29-11-19	..	Clear	Normal	Not increased	+	..	—
5	28-12-19	..	..	..	..	+	Slight	Positive.
8	26-12-19	..	..	Increased	..	++	"	Negative.
9	26-12-19	..	..	..	..	++	(More marked than Case 8)	Negative.
16	27-12-19	..	..	Much increased	..	++	Marked	Negative.
17	26-12-19	..	..	Slightly increased	..	++	"	Negative.

TABLE IV.

Table showing the result of the examination of urine from tetanic encephalitis cases Karachi.

Case No.	Date of examination.	Appearance.	Specific gravity.	Reaction.	Albumen.	Cast.	Sugar.	REMARKS.
5	30-12-19	..	1.018	Faintly acid	Present	Nil	Trace	Pus cells present.
8	31-12-19	..	1.008	Faintly acid	Nil	Nil	Nil	....
9	26-12-19	..	1.020	Acid	Nil	Nil	Nil	Phosphates present.
17	30-12-19	..	..	Acid	Nil	Nil	Trace	Phosphates present.

An examination of Table II brings out some interesting points.

(1) With the exception of Case 5, whose symptoms may have been entirely due to syphilis, all of the patients had a moderate degree of leucocytosis during their illness. This is a condition which, as far as we are aware, has been rarely encountered in cases of lethargic encephalitis in Europe and America. The leucocytosis persisted during convalescence, and was seen in patients who were well on the road to complete recovery.

(2) Bearing in mind that the average differential blood count in healthy Indians is as follows:—\* neutrophile polymorphs 67 per cent, eosinophiles 2 per cent, large monocytes 9 per cent, small monocytes 22 per cent, it will be seen that there has been a definite increase of the polymorphonuclear cells in our cases.

(3) The number of cases has not been large, and blood counts have been made infrequently; the figures, however, suggest that in the early stages of the disease polymorphs are present in comparatively large numbers. As the patient improves, their percentage decreases while that of the small monocytes increases.

Leaving Case 5 (syphilis) out of account, the lowest percentages of polymorphs are recorded in cases 8, 10 and 17 on the 32nd, 29th and 34th days of illness respectively, and these are the very cases where improvement has been most marked; while, at the same time, there has been practically no alteration in the total leucocyte count. In Case 9, where improvement has been slight, the high percentage of polymorphs still persists (32nd day). The increasing percentage of eosinophiles may possibly be evidence of the progress of a regenerative process.

In Table III three points are brought out:—

- (1) The cerebrospinal fluid was always clear and generally under increased pressure.
- (2) No pleocytosis was observed.
- (3) Globulin was present in excess.

#### CULTURES AND ANIMAL EXPERIMENTS.

Blood and cerebrospinal fluid were taken from cases 5, 8, 9, 10, 16 and 17 on the 33rd, 23rd, 23rd, 29th, 11th and 17th days of the disease respectively. No growth was obtained in any instance on the ordinary laboratory media. Tubes containing Ringer's solution, inactivated

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\* The average of more than 100 counts made by one of us. (G. C. M.)



rabbit's serum and 0·3 per cent agar were planted anaerobically (in Buchner's tubes) and under increased carbon dioxide tension with negative results.

A series of guinea-pigs were inoculated intraperitoneally with 2 to 5 c.c. of blood and cerebrospinal fluid from these cases, and another series intracranially with approximately 0·5 c.c. of centrifuged spinal fluid. Five c.c. of the spinal fluid from Case 16 was inoculated intravenously into a monkey. None of these animals have so far shown any sign of illness. Centrifuged specimens of urine were injected into the peritoneal cavity of guinea-pigs without any effect.

Post-mortem examinations have not been obtainable.

#### CONTAGIOUSNESS OF LETHARGIC ENCEPHALITIS AND ITS RELATION TO INFLUENZA AND POLIOMYELITIS.

As the accompanying map shows, the cases have appeared in many different parts of the city. No two cases have occurred in the same house, or even in neighbouring houses. Careful enquiry has failed to elicit any evidence that the disease has been transmitted directly from one case to another.

The relation between lethargic encephalitis and influenza is also obscure. Great stress has been laid by certain epidemiologists on the appearance of that disease during an influenza prevalence. Sachs<sup>(1)</sup> and Tucker<sup>(2)</sup> report a number of cases in which an attack of influenza was followed by lethargic encephalitis after an interval of several weeks. Milian<sup>(3)</sup> reports a fatal case occurring during convalescence from influenza with broncho-pneumonia. On the other hand, many observers have recorded cases in which there was no history of influenza.

We have been able to trace the histories of ten of the Karachi cases. None of them had suffered from influenza in 1918 or 1919. The wife and child of one had influenza in the present epidemic; the mother and sister of another gave a history of influenza in 1918. In none of the remaining 8 cases was there any influenza in the family or in the house, and in two instances of these influenza had not occurred even in the neighbourhood.

Further, during the epidemic of influenza in Karachi in the latter part of 1918 and in Calcutta in the early part of 1919, a large number of cases of influenza with meningeal symptoms and drowsiness were observed. Cranial nerve paralysis did not appear in any of these, although this condition is one of the most striking features of encephalitis.

In the present epidemic in Karachi, meningeal symptoms in influenza have been very rare, still lethargic encephalitis has made its appearance. The influenza has been chiefly of the pneumonic and broncho-pneumonic types, yet none of our cases of lethargic encephalitis have shown any catarrhal symptoms or signs of lung affection.

It has been generally stated that the blood of patients with influenza not complicated with pneumonia, has shown leucopenia.

Von Becher (quoted from *Medical Science, Abstracts and Reviews*, Vol. I, No. 1) 'examined the blood of 100 cases with the following results:—

(1) In 29 cases of uncomplicated influenza or with tracheobronchitis only, 27 showed leucopenia.

(2) Of 29 cases with broncho-pneumonia, 22 showed leucopenia.

(3) Of 35 cases of croupous pneumonia alone or combined with broncho-pneumonia, 20 showed leucocytosis and 15, of which 12 were fatal, leucopenia.'

None of our cases of lethargic encephalitis had either bronchitis or pneumonia, but all showed a moderate degree of leucocytosis with an increase of the polymorphonuclear cells.

An enquiry by the Local Government Board (London) and the Medical Research Committee<sup>(4)</sup> on lethargic encephalitis showed '(a) that in its essential primary features the illness had a characteristic and constant symptom series of its own, (b) that between this symptom series and that of the rare forms of poliomyelitis, with which alone it could be confused, the clinical differences were more marked than the resemblances. The results of the epidemiological inquiries were to the effect that encephalitis lethargica was not a form of acute poliomyelitis, and that its presence and epidemic prevalence depended on conditions other than those necessary for the presence and epidemic prevalence of that disease.'

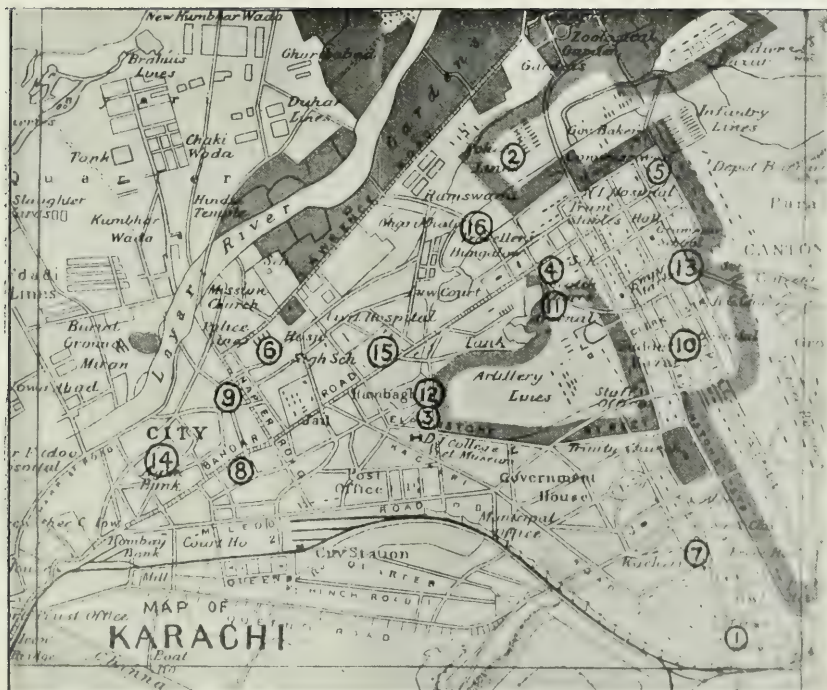
In our cases the symptoms corresponded closely with those described for lethargic encephalitis and differed from the commoner forms of poliomyelitis not only in the symptomatology but also in respect of age incidence, mortality and the cytology of the cerebrospinal fluid.

Thus:—

(1) Seventeen of the 20 cases occurred in persons over 15, and none in children under 10 years of age.

(2) The mortality was very high—35 per cent of 20 cases.

PLATE LIV.



Map of Karachi showing the distribution of 16 cases of lethargic encephalitis occurring in the city.

R. H. MALONE and G. C. MAITRA —Lethargic Encephalitis in Karachi.



- (3) All of the cerebrospinal fluids examined by us were clear and showed no leucocytosis although the Nogouchi test for globulin was positive.

#### SUMMARY.

(1) Cases of lethargic encephalitis have occurred in Karachi during the present influenza prevalence.

(2) The history of 10 cases has been obtained. None of them records an attack of influenza, and in one case only was there influenza in the family during the present epidemic.

(3) No evidence has been brought forward that the disease is contagious and no light has been thrown on its etiology.

(4) Attention is directed to the results of the laboratory examination of the blood and cerebrospinal fluid.

Moderate leucocytosis has occurred in all of our cases with a definite increase of polymorphonuclear cells. The total leucocyte count varied but little during the illness, but as the patient's condition improved there was noted a decrease in the polymorphonuclear neutrophiles and a relative increase in the eosinophiles and small monocytes.

The cerebrospinal fluid of every case was clear but contained an excess of globulin. There was no increase of cells.

Our thanks are due to Col. McKechnie, I.M.S., for placing the Laboratory, 37 Indian General Hospital, at our disposal and to Dr. Shroff, Health Officer, Karachi, Lieut.-Col. Stephen, I.M.S., and Captain Wrench, R.A.M.C., for valuable help in many ways.

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# THE PREPARATION OF A CULTURE MEDIUM SUITABLE FOR THE GROWTH OF ORGANISMS USED AS VACCINES.

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JUDGING by the multiplicity of methods at present in use for the growth of *B. typhosus* and *B. cholerae* on the large scale, it is evident at once that a culture medium, satisfactory in all respects, has not yet been obtained.

This is no doubt mainly due to the fact that it is only in recent years, that owing to the large demand for vaccine for troops, it has been realised that there is a wide field for research in bacterial nutrition.

It is a problem which has not greatly appealed to the bacteriologist and which has been equally neglected by the biochemist. This in some measure has been due to the difficulty in estimating with any degree of rapidity and even accuracy the numbers of organisms grown.

Too much attention has been paid merely to appearance of growth rather than to actual quantity and because of this a great deal of work has remained mainly of academic interest.

Recent research (Brown, *Indian Journal of Medical Research*, July 1919) has, however, solved this problem so that a close estimation of numbers of bacteria grown can be made with ease by a well-trained laboratory attendant. This being so, it is at once obvious that culture media can now be classified with some degree of accuracy according to their nutritive value, a point which previously was difficult to ascertain. This simple method of estimation of growth has also rendered it possible to follow up lines of attack in the preparation of culture media, which would otherwise have been almost certainly neglected owing to

the immense amount of labour involved and the inconclusiveness of the results obtained.

In certain laboratories the principal method for the preparation of culture medium consisted in the production of a clear broth in which the pure organism was grown. In the case of *B. typhosus* after a certain period of incubation the broth cultures were killed by being heated in a water bath for an hour and ten minutes at 53°C. They were then cooled, tested for sterility and a certain amount of preservative added. The numbers of organisms present in 1 c.c. of culture medium were then estimated by Wright's method. (See H. W. Grattan and A. L. Webb, *Journal, R.A.M.C.*, Vol. XIII, 1909, p. 62). The necessary dilution to standard strength was effected and sterility again tested.

During the war (1916) when a mixed typhoid paratyphoid vaccine was substituted for the previous typhoid one, the *B. typhosus* was still a broth culture but the paratyphoids were grown on agar and added afterwards to the fluid culture of *B. typhosus* in the proportion 2 parts *B. typhosus*, 1 part *B. para A* and 1 part *B. para B*. At the Central Research Institute, Kasauli, early in 1915, an agar typhoid vaccine was made and used with satisfactory results. The medium in this case was Witte's peptone mutton broth agar. The next great advance took place as the result of Douglas's work on the preparation of nutrient broth by using the products of tryptic digestion of meat. (Douglas, *Lancet*, Vol. II, 1914, p. 891.) This method gave greatly enhanced growths both of *B. typhosus* and *B. cholerae*, as compared with those previously obtained on peptone media.

Modifications of Douglas's original process had to be adopted in this country, and mutton was used as the substrate for digestion, and the pancreatic extract was obtained from sheep and goats. Agar medium prepared from broth obtained in this way was found to be exceedingly nutritive both for *B. typhosus* and *B. cholerae* and this modified process was adopted in the Central Research Institute, as a routine method for the preparation of culture medium towards the end of 1915.

In all the foregoing media, meat in some form is the essential basis. Since, however, all recent work on bacterial nutrition has shown that complex protein groups cannot be easily assimilated but that some preliminary breaking down of the molecule is essential, it would seem possible that nutrient material could be obtained from other sources than meat. Experiments with this point in view were accordingly devised and the results form the basis of the present communication.



In the preparation of vaccines on a large scale many factors have to be borne in mind, but perhaps the two main essentials are cheapness of materials employed and luxuriance of bacterial growth.

Meat suitable for culture media as a rule is not expensive but it cannot be stored in large quantities, so that fluctuation in amounts supplied may at any time be a serious matter. Hence the possibility of using some substance, which can be stored in bulk, is an important consideration.

Previous work (Norris, *Indian Journal of Medical Research*, October 1918, and April 1919) suggested two such products available on a sufficiently large scale, namely, groundnut cake (*arachis hypogæa*) and caseinogen. Both substances were thoroughly well tested in the laboratory, and finally caseinogen was adopted in preference to groundnut press-cake.

Groundnut press-cake is easily procurable from prisons in this country, the expression of the oil constituting a well-established industry.

The main use of the press-cake thus obtained is as a cattle food, and in appearance it is a dirty brown looking mass. It may, of course, be purified by further treatment and a fine white flour obtained. This, however, adds to the cost and labour of manufacture.

In any case the press-cake as supplied needs grinding before use, thus involving a corresponding amount of time and labour. Even, however, when made up into culture medium in its crude state, very satisfactory results are obtained as may be seen from the following table:—

TABLE I.

*Showing yield of B. typhosus from agar containing as basis the products of tryptic digestion of groundnut press cake.*

(i) For 24 hours.

(ii) For 6 hours.

(iii) Douglas medium from six hours digestion for comparison.

*Measured in 100 million per sq. c.m.*

Percentage press-cake as substrate.	i	ii	iii
10	42.75	53.45	26.72
	47.50	53.45	28.50
	71.28	30.52	25.62
	69.89	47.50	35.62
	69.89	30.52	37.50
	42.75	28.55	28.50

Made use of in this way, it gives rather a dirty looking culture medium and samples vary somewhat with regard to their nutritive value.

In view of the fact, however, that caseinogen is rapidly becoming a valuable product, further experimentation with a more purified press-cake is clearly indicated.

Caseinogen has the advantage of being a much cleaner product. It is supplied in the form of a granular powder and does not require any further treatment before use.

It gives a cleaner looking medium than crude groundnut press-cake and rather more consistent results when tested for nutritive efficiency, as may be seen when the following table is compared with the previous one.

TABLE II.

*Showing yield of B. typhosus from agar containing as basis the products of tryptic digestion of caseinogen.*

- (i) For 24 hours.
- (ii) For 6 hours.
- (iii) Douglas medium from 6 hours digestion for comparison.

*Measured in 100 million per sq. c.m.*

Percentage caseinogen as substrate.	i	ii	iii
	71.26	53.45	26.72
	71.26	69.89	28.50
10	69.89	53.45	35.62
	69.89	71.26	35.62

Neither caseinogen nor crude groundnut press-cake are of any value as nutrient media before hydrolysis (Norris, *Indian Journal of Medical Research*, April 1919). For the preparation of culture media suitable for the growth of organisms used as vaccines, where enzymatic hydrolysis is employed as a means of obtaining the necessary degradation products, it must be borne in mind that the shorter the period of digestion the less likelihood of contamination and the less necessity for the use of antiseptics.

It is practically essential to use either chloroform or toluene as an antiseptic for any period of digestion of twenty-four hours or more for caseinogen, crude groundnut press-cake or meat at 37°C. This at once raises serious objections to such a process for large scale work on the score of cost alone. In this connection caseinogen appeared to possess

an advantage over groundnut press-cake as during a six-hour hydrolysis with pancreatic extract, the yield of amino-acids as demonstrated by Sørensen's method is consistently greater with the former substance than with the latter—the two figures approximating after a hydrolysis of 24 hours. This is demonstrated in the following table:—

TABLE III.

*Showing comparative yield of amino-acids, as demonstrated by Sørensen's method of estimation, resulting from the tryptic digestion of caseinogen and crude groundnut press-cake during a period of six hours and twenty four hours.*

Time.	10 % caseinogen as substrate for hydrolysis.	10 % crude groundnut press-cake as substrate for hydrolysis.
	Yield of amino-acids expressed in c.c.s N/10 NaOH per 10 c.c.s substrate.	Yield of amino-acids expressed in c.c.s N/10 NaOH per 10 c.c.s substrate.
6 hours.	4.5	0.7
	4.5	0.8
	4.3	1.0
	4.4	1.5
	4.7	1.5
24 hours.	7.7	7.4
	9.5	7.0
	7.7	7.0

Results obtained so far do not seem to show that bacterial growth increases correspondingly with an increased production of amino-acids. For example, a ten days' digestion of caseinogen showed an amino-acid production corresponding to 24.25 c.c.s N/10 NaOH, but the bacterial growth subsequently obtained was no greater than that from a six hour caseinogen digest showing only 6.5 c.c.s N/10 NaOH. Similarly in the case of crude groundnut press-cake an amino-acid production corresponding to 11 c.c.s N/10 NaOH gave no better subsequent bacterial growth than one showing 7.0 c.c.s. Hence in the above table, although this difference in the amino-acid production at the beginning of the hydrolysis may serve as an indication that subsequent bacterial growth will also differ, it does not by any means necessarily account for this difference, otherwise one should be able to show that growth increases with the amino-acid production which is not the case as has already been noted.

This is again a point requiring further investigation as it might be possible so to arrange the conditions of hydrolysis, that the formation of substances necessary to bacterial growth might be brought about apart from any special increased concentration of amino-acids. Meantime, Sørensen's method of estimation of amino-acids serves as a very useful indication that hydrolysis has taken place, although when more information as to the particular substances required in bacterial nutrition has been obtained, it may be possible to replace it.

*Method of Preparation of Caseinogen Medium.*

It is obvious, from the foregoing facts, that caseinogen may be substituted for meat in the preparation of culture medium with obvious advantages.

The details of the mode of preparation of caseinogen medium are as follows :—

Twenty-five litres of water are boiled in a large tub of about 70 litres capacity, and 200 grammes commercial anhydrous sodium carbonate are added. 10 lb. of caseinogen are then gradually shaken in and the whole brought to the boiling point.

Twenty-five litres of cold water are then added and the reaction tested with litmus paper which should indicate alkalinity.

The temperature is also taken and, if not more than 48°C, 2 litres of pancreatic extract (for preparation see Cole, *Lancet*, July 1, 1916) are added. A sample is then taken for estimation of amino-acids by Sørensen's method and the remainder is left to digest 3½—4 hours at 37°C.

After this it is boiled and filtered through muslin and the filtrate measured.

A sample is again taken for estimation of amino-acids by Sørensen's method and these should show an increase equivalent to about 3 c.cs, N/10 NaOH per 10 c.cs, broth.

Following the method previously in use in the Institute, 2·5 grammes NaCl and 0·125 grammes Ca Cl<sub>2</sub> are then added to each litre and the whole is sterilised at 115°C for one hour.

The broth is again filtered and 100 c.cs together with 4 grammes desiccated agar (for preparation see Cunningham, *Indian Journal of Medical Research*, April 1919) put into each round whisky bottle which are found to be much cheaper than Roux flasks and afford a large surface. These are then sterilised at 120°C for 2 hours.

The above quantities should give about 450 bottles.

The advantages of this method are perhaps better seen when compared with the previous one in use.

Meat medium.	Caseinogen medium.
1. 2 lb. fat free mutton minced.	1. 25 litres boiling water, 200 grammes ordinary dry sodium carbonate.
2. 2 lb. plus 2 litres water placed in 3 litre tin pot.	2. Add slowly 10 lb. caseinogen and bring to boiling point.
3. Autoclave at 130°C for 1 hour.	3. Add 25 litres cold water.
4. Keep overnight but, if urgently wanted, cool to 45°C and continue as below.	4. Test reaction with litmus paper which should indicate faint alkalinity.
5. Test amino-acidity by Sørensen's method.	5. Take temperature if not more than 48°C add 2 litres pancreatic extract.
6. Add 40 c.c. pancreatic extract per litre.	6. Estimate amino-acidity by Sørensen's method.
7. Digest 4 hours at 37°C.	7. Leave digesting 3½-4 hours at 37°C.
8. Take culture on agar and broth and repeat amino-acidity estimation.	8. Boil and filter through muslin and measure filtrate.
9. Filter through muslin to remove fat.	9. Estimate amino-acidity as before.
10. Add 1 c.c. glacial acetic acid per pot.	10. To each litre add 2·5 grammes NaCl and 0·125 grammes CaCl <sub>2</sub> .
11. Sterilise by heating up to 110°C and then cool.	11. Sterilise at 115°C for one hour.
12. Filter to remove mince.	12. Filter and put 100 c.c. filtrate and 4 grammes desiccated agar into each whiskey bottle.
13. Make slightly alkaline to litmus, add 2·5 grammes NaCl and 0·125 grammes CaCl <sub>2</sub> per litre.	13. Sterilise 2 hours at 120°C.
14. Sterilise at 115°C for one hour.	14. Roll. These quantities give 450 bottles. 2 lbs. caseinogen give 80-85 bottles.
15. Filter through filter paper.	
16. Put 100 c.c. broth into each whiskey bottle.	
17. Sterilise at 120°C for 1 hour.	
18. Add 4 grammes desiccated agar to each bottle.	
19. Sterilise at 120°C for 1 hour.	
20. Roll. These quantities give 15 or 16 bottles.	

By comparing the above processes it is seen that a great saving, both in time and labour, is achieved apart from the fact that at present rates caseinogen medium actually works out 7·8 times cheaper than that prepared from meat.

It has other advantages in that autoclaves are more available for other work, such as sterilisation of saline which is by no means a small factor in a large vaccine-producing Institute.

Another great advantage is the fact that caseinogen appears to keep indefinitely, and hence a large or small amount may be made up into medium daily, whereas with meat it has either to be used up at once or

wasted. Furthermore the residue in the case of the tryptic digestion of meat is much greater than that from an equivalent tryptic hydrolysis of caseinogen.

Broth prepared from caseinogen does not appear to retain its nutritive power as long as that obtained from meat although further experiments are required on this point. It has also not been possible so far to prepare a perfectly clear broth from caseinogen so that its main use is in the preparation of solid media for vaccine purposes.

There is one point in the preparation of caseinogen medium which requires care and that is the adjustment of the reaction before hydrolysis. This is only of importance in dealing with a staff where a biochemist is not available.

The following table designed for use by an Indian staff has been found to work well and is given here in the possibility of saving trouble for others.

*Directions for Preparing Caseinogen Broth.*

Pancreatic extract should have a slightly alkaline substrate in order to obtain a good hydrolysis. The best working reaction is given by 0.4 per cent ordinary dry sodium carbonate or 0.8 per cent ordinary washing soda.

The following amounts give those usually employed in the Central Research Institute:

TABLE IV.

Caseinogen, lbs.	Total water litres.	Dry sodium carbonate, grammes	Washing soda, grammes.	Pancreatic extract, c.c.s.
2	10	40	80	400
2½	13	52	104	520
3	15	60	120	600
4	20	80	160	800
5	25	100	200	1,000
6	30	120	240	1,200
7	35	140	280	1,400
7½	38	156	312	1,520
10	50	200	400	2,000

If the caseinogen is an acid sample add half the above amounts of either alkali more and test reaction with litmus paper which should show faint alkalinity. If still acid add half the same amount again. A sample of every new consignment of caseinogen should be tested for acidity.





# PRELIMINARY NOTE ON A METHOD OF UTILISING THE NATURAL AMBOCEPTOR IN HÆMOLYTIC SERA IN THE WASSERMANN REACTION.

BY

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THE existence in certain human sera of a natural amboceptor for sheep's corpuscles is now well known. This declares itself during a Wassermann test by causing hæmolysis in control tubes to which no amboceptor has been added and in which no hæmolysis should occur. If hæmolysis occurs and the remaining controls are correct, it indicates the presence in the serum of some substance which is functioning as an amboceptor for sheep's corpuscles. If this is not allowed for, it may conceal a positive Wassermann reaction and end in the unfortunate result of a false negative being reported.

The usual methods of obviating this difficulty are :—(i) Noguchi's method of substituting an antihuman hæmolytic system for the original Wassermann antisheep hæmolytic system, and (ii) the method after Castellani of extracting the natural amboceptor for sheep's erythrocytes by incubation at room temperature with these, and pipetting off the supernatant fluid and using the patient's serum, thus treated, for the test by the ordinary Wassermann technique instead of the original serum. Method (i) is inconvenient for various reasons, more particularly on

account of the difficulty of obtaining an antihuman amboceptor serum of high titre. We have used method (ii) with satisfactory results and have recently employed it as a test to gauge the results of method (iii) which we describe here.

The procedure is extremely simple. It consists in the utilisation of the natural amboceptor, instead of its removal, and has the advantage of automatically titrating it at the same time.

Let us suppose 0·5, 0·6, 0·7 c.c. . . . . etc., up to 1 c.c. of a 1 in 10 dilution are the doses of serum employed in the test, and that 0·5 c.c. of a 1 in 50 dilution is the titrated dose of the antishæep amboceptor.

Then put up a set of tubes as follows :—

- |     |   |            |          |
|-----|---|------------|----------|
| (1) | 0·5 c.c. serum +                                  | complement | + cells. |
| (2) | 0·6 c.c. serum +                                  | do.        | + do.    |
| (3) | 0·7 c.c. serum +                                  | do.        | + do.    |
| (4) | 0·8 c.c. serum +                                  | do.        | + do.    |
| (5) | 0·9 c.c. serum +                                  | do.        | + do.    |
| (6) | 1·0 c.c. serum +                                  | do.        | + do.    |
| (7) | No serum + 0·5 c.c. of amboceptor (1/50) + cells. |            |          |

Thus amboceptor is only added to the last tube which contains no serum. Incubate for half an hour at 37°C. Now each of these tubes (in the case of a naturally hæmolytic serum) contains a hæmolytic system and hæmolysis will therefore occur to a greater or less degree. Note that tube which has hæmolysed to the same extent as tube 7 (the standard). This is best done by centrifugalising after the incubation and comparing the colour of the supernatant fluid. Let us suppose it is tube 4. Now tube 4 contains 0·8 c.c. of serum (1/10). Therefore 0·8 c.c. of serum (1/10) contains 0·5 c.c. of amboceptor (1/50). Then to perform the complement fixation test put up three tubes as follows :—

- |     |                                  |            |               |
|-----|----------------------------------|------------|---------------|
| (1) | 0·8 c.c. serum +                 | antigen    | + complement. |
| (2) | 0·8 c.c. serum +                 | no antigen | + complement. |
| (3) | No serum + antigen + complement. |            |               |

Incubate for an hour at 37°C. Then add the corpuscles to all, but add amboceptor only to tube 3 and incubate again. Hæmolysis should occur in all three tubes in the case of a non syphilitic serum. Inhibition in tube (i) means a positive result. It is of course understood that a complete series of controls was put up in the first Wassermann test. We have thus titrated the natural amboceptor and have avoided adding more. The only difference in principle from the usual Wassermann technique is

that the amboceptor is present in the tube during the first incubation, whereas in the normal Wassermann reaction it is not. In practice, this makes no difference nor *ex hypothesi* would any difference be expected.

We have not had an opportunity as yet of working the method out fully, but in a series of thirty naturally hæmolytic cases recently met with, *the results obtained corresponded exactly with those arrived at by extracting the amboceptor*, and we think that our method is simpler and yet more accurate in that it enables us to estimate the amount of natural amboceptor present, and to utilise it.

# BIONOMICS OF HOUSEFLIES.

## I.

### OUTDOOR FEEDING HABITS OF HOUSEFLIES WITH SPECIAL REFERENCE TO MUSCA PROMISCA (ANGUSTIFRONS ?).

BY

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[Received for publication, October 30, 1919.]

THE outdoor feeding habits of houseflies have not yet been thoroughly investigated. The chief aim of this paper is to examine which of the species of *Musca* are intimately connected with fresh human faeces as far as their feeding is concerned. Since such fresh faeces are usually found infected in times of epidemics of cholera, typhoid, etc., and are generally voided in such places as are easily accessible to houseflies, it is very important to know which of the species of *Musca* are first attracted to feed on such fresh faeces.

The species of *Musca* may be conveniently divided into two groups: one of which consists of the hematophagus species, such as *M. bezzii*, etc., which are generally found associated with cattle; while the other group contains such species as frequent dwelling houses and are more or less found with man. The present paper takes notice of the species of the second group only.

The expression 'fresh human faeces' requires some explanation. It is meant to denote fresh faeces in isolated patches and in a liquid or semi-liquid condition. Both these conditions are essential to arrive at the conclusions stated in this paper as far as feeding of *M. promisca* is concerned. Human faeces heaped together or mixed with other things seem to be quite different, as they are found to attract other species than *M. promisca*.

The tables which have been attached to this paper tabulate the results of the experiments carried out at two different stations: Bombay and Nagpur.

TABLE No. I.

*Showing frequency of the species of Musca under different situations.*

Species.	Sweetmeat shops.	Residential houses.	Eating houses.	Fresh human faeces.	Waste food.
M. divaricata (nebulo) ..	452 ; 70%	236 ; 77%	506 ; 81%	84 ; 11%	396 ; 73%
M. multispina ..	73 ; 11%	40 ; 13%	78 ; 13%	13 ; 2%	55 ; 10%
M. promisca ..	125 ; 19%	30 ; 10%	39 ; 6%	670 ; 87%	93 ; 17%
TOTAL ..	650	306	623	767	544

The above table shows the frequency of those species of *Musca* which are found in houses, shops, etc.

If the relative frequency of these three species in different situations be taken into consideration, it will be seen that *M. divaricata* is to be found in great numbers in sweetmeat shops, hotels, houses, but in considerably less numbers on fresh human faeces. On the other hand, however, *M. promisca* tops the list as far as fresh human faeces are concerned. It constitutes 87 per cent of the total ; while the other two species together contribute 13 per cent only.

That the relative proportions of these three species in different situations and under different conditions of weather remain more or less constant throughout a year will be clearly illustrated by the following three tables :—

TABLE II.

*Showing frequency of these three species in a dry season before the rains.*

Species.	Sweetmeat shops.	Residential houses.	Eating houses.	Fresh human faeces.	Waste food.
M. divaricata ..	99 ; 69%	23 ; 79%	124 ; 84%	39 ; 17%	103 ; 84%
M. multispina ..	8 ; 6%	4 ; 14%	12 ; 8%	9 ; 4%	9 ; 7%
M. promisca ..	36 ; 25%	2 ; 7%	12 ; 8%	178 ; 79%	11 ; 9%
TOTAL ..	143	29	148	226	123

TABLE III.

*A wet season.*

Species.	Sweetmeat shops.	Residential houses.	Eating houses.	Fresh human faeces.	Waste food.
<i>M. divaricata</i> ..	52 ; 48%	84 ; 67%	79 ; 68%	28 ; 15%	249 ; 71%
<i>M. multispina</i> ..	9 ; 8%	18 ; 14%	13 ; 11%	3 ; 2%	38 ; 11%
<i>M. promisca</i> ..	48 ; 44%	24 ; 19%	24 ; 21%	160 ; 83%	65 ; 18%
TOTAL ..	109	126	116	191	352

TABLE IV.

*In a dry season after the rains.*

Species.	Sweetmeat shops.	Residential houses.	Eating houses.	Fresh human faeces.	Waste food.
<i>M. divaricata</i> ..	301 ; 76%	129 ; 85%	303 ; 84%	17 ; 5%	44 ; 64%
<i>M. multispina</i> ..	56 ; 14%	18 ; 12%	53 ; 15%	1	8 ; 11%
<i>M. promisca</i> ..	41 ; 10%	4 ; 3%	3 ; 1%	332 ; 95%	17 ; 25%
TOTAL ..	398	151	359	350	69

It will be seen by comparing one table with the other that though the relative proportions of the frequency of these three species remain more or less the same, there are, however, certain individual variations. In the wet season, *M. promisca* is found in greater numbers in sweetmeat shops than in the other two dry seasons of the year ; while its prevalence on fresh human faeces is at its maximum after the monsoons. If there is any chance of this species carrying infection from infected fresh human faeces to sweetmeats, it is most likely to occur in a wet season unless the increase observed be only an apparent one due to

the decrease in the numbers of the other species frequenting these substances.

It has been stated above that the human fæces, heaped together or mixed with earth or garbage, or allowed to get dry, attract a different species of *Musca* in greater numbers. The following table will make this clear:—

TABLE V.

Species.	Human fæces mixed with clay (trenches).	Human fæces mixed with food.	Human fæces once dried but soaked in rain water.
<i>M. promisca</i> ..	4	9	52
<i>M. divaricata</i> ..	584	160	162
TOTAL ..	588	169	214

It will be found from the above table that the number of *M. promisca* falls very low as the human fæces become mixed with other things: while that of *M. divaricata* increases. Thus it can be safely inferred that fresh human fæces in isolated patches are attractive to *M. promisca*, but not so to *M. divaricata*; while, on the other hand, human fæces, mixed with earth or garbage or heaped together, ceases to be attractive to *M. promisca* but becomes much more attractive to *M. divaricata*.

Table VI will show that *M. promisca* begins to fall in numbers as fresh human fæces become stale day by day and that the same patch of the human fæces, when stale, will attract a great number of the other species.

TABLE VI.

Species.	Fresh human fæces exposed for twenty-four hours or less.	Fresh human fæces exposed for two or three days or more.
<i>M. promisca</i> ..	1,463	38
<i>M. divaricata</i> ..	14	155
TOTAL ..	1,477	193



## CONCLUSIONS.

(1) *M. divaricata* (*nebulo* ?) is most abundantly found in dwelling houses, sweetmeat shops, hotels, on stale human faeces and on human faeces mixed with other matter.

(2) *M. promisca* (*angustifrons* ?) is mostly attracted to fresh human faeces. It is also found in sweetmeat shops, though in comparatively small numbers.

# BIONOMICS OF HOUSEFLIES.

## II.

### ATTRACTION OF HOUSEFLIES TO DIFFERENT COLOURS.

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It has been stated by certain writers on this subject that houseflies are not at all attracted to colour. Recently Lodge (*Bulletin of Entomological Research*, Vol. IX) has again emphasised this fact. But my experiments seem to show that various colours do have different degree of attraction for these insects.

Two series of experiments were undertaken. One of them was carried out at night, when artificial lights were exposed. The second series was performed by day, when different coloured tanglefoot papers were exposed.

The results of these two series correspond closely with each other.

#### I. EXPERIMENTS DONE AT NIGHT.

For these experiments several small tin lanterns were made in such a way that three sides as well as the bottom and the top of each lantern were made light-proof, while to the fourth side was attached a small piece of glass of particular colour. Special care was taken to have the same intensity of light source in every case by burning a small piece of candle in each lantern. A single sheet of a sticky paper was kept with each lantern to catch those flies which were attracted to that particular colour and thus I obtained an index to the numbers of flies attracted to it.

For every experiment flies were caught in large numbers by day and were let loose at the time of the night experiment.

These night experiments are described under three headings :—

(a) The coloured lights were placed on the circumference of an imaginary

circle at the centre of which flies were liberated : (b) the lights were placed one behind the other in a straight line : and (c) obstacles were interposed between certain of the lights and the flies liberated, so that only some of the lights were directly visible to them.

(a) Lights arranged circumferentially.

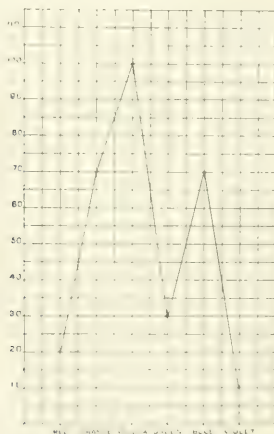
In every experiment lights were kept in such a way that they were visible directly from the point at which the flies were liberated. This point was distant about 10 feet from all the lights. The results of all these experiments are given in the table below, the colours being taken in the order of the spectrum :—

TABLE I.

Colour.	No. of experiments.	Flies caught.	Average No. of flies attracted.
Red .. ..	8	154	19
Orange .. ..	3	222	74
Yellow .. ..	9	923	103
Green .. ..	7	203	29
Blue .. ..	10	655	66
Violet .. ..	4	50	13

This same table can be shown in a graph form :—

CHART I.



Number of flies attracted.

From the above table as well as from the curve, it will be seen that the flies have the least attraction for the colours of the extremities of the spectrum, *i.e.*, red and violet; while on the other hand, yellow, orange and blue are the most attractive to these flies.

(b) Lights were placed one behind the other in a straight line and directly visible.

These lights were placed at a distance of 10 feet from each other. Flies were liberated from a spot which was in a straight line with the lights and which was also 10 feet away from the nearest light. The following table will show the results of these experiments:—

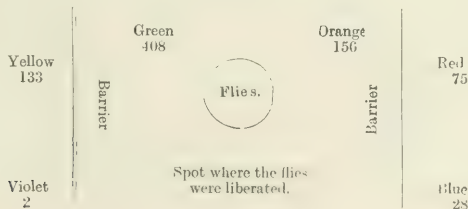
TABLE II.

Colour.	No. of flies attracted.	M. DIVARICATA.		M. PROMISCA.	
		Females.	Males.	Females.	Males.
Yellow ..	157	139	5	13	..
Blue ..	56	18	3	5	..
Green ..	36	33	..	3	..
Red ..	8	8	..	..	..

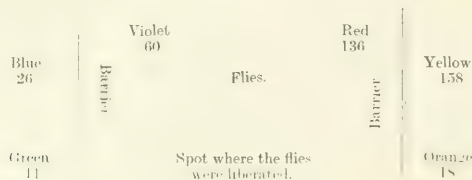
The order of the lights was, in all the experiments, such that red was the nearest and yellow the farthest from the spot from where the flies were liberated.

It will be seen from the above table that houseflies seem to have colour preferences, otherwise they would not have passed by so many colours to reach the yellow.

(c) Obstacles were interposed so that some of the lights were not directly visible from the place where the flies were let loose. The following diagrammatic arrangement of the lights and the place of liberation of the flies will give an idea of the experiment. The number of the flies attracted is given with each colour.



In the second series of the experiments the arrangement of colours was altered :—



In the third series none of the lights were directly visible to the flies, as they were kept behind a barrier. Each light was at a distance of two feet from the other. The results were as follow :—

Yellow 137	Red 39	Blue 121	Orange 64	Green 39	Violet 28	as the lights were placed in a row.
---------------	-----------	-------------	--------------	-------------	--------------	--

In the first and the second series of the experiments, when some of the lights were screened from view, flies were attracted in great numbers to those lights which were directly visible. In the third series when all the lights were hidden, the order of attraction was the same as described in Table I.

Thus it may be presumed that the flies in the absence of their favourite colours will be attracted to any colour which is directly visible to them.

*Flies other than houseflies attracted to different colours.*

The flies other than *Musca* were few. But in spite of this one may say that *Calliphora* (green as well as blue bottles) showed certain marked preferences to different colours, more or less similar to those found among the houseflies. The following table will show this :—

TABLE III.

Colour.	No. of <i>Calliphora</i> attracted.
Red .. ..	4
Orange .. ..	6
Yellow .. ..	24
Green .. ..	7
Blue .. ..	16
Violet .. ..	2
TOTAL .. ..	59

Thus it will be seen that yellow has still the greatest attraction for other flies such as green and blue bottles ; and red and violet the least.

## II. EXPERIMENTS BY DAY.

The experiments detailed above, it may be objected, were carried on in the night time when flies are supposed to be lethargic, and may respond to any colour when roused from this condition. It may be said, in reply to this objection, that flies would not show continuously the same kind of response, night after night, to a particular colour in a definite way. But to remove this objection altogether, certain experiments were carried out by day to corroborate the results of those of the night time.

It will be shown presently that the results of the experiments of the day time are exactly similar to those of the night. Recently Lodge has stated, in one of the issues of the *Bulletin of Entomological Research*, Vol. IX, that flies do not show any colour preference at all. She has tried different coloured chalks and other things mixed with the same food which is supposed to be attractive to flies. She has exposed these colours and found flies indiscriminately all over the various colours. She concludes, therefore, that flies have no colour preference at all. Hindle, in the *Journal of Hygiene*, Vol. XIV, had come to the same conclusion before Lodge. His method was quite different. He painted bands of different colours on the same piece of a card-board which had been washed with a sticky substance. He likewise found flies indiscriminately all over the colours and hence his conclusion was that flies had no colour preference.

The conclusions deduced from the experiments to be described below are quite different from those of the above workers. The method which I have used is as follows :—Pieces bigger than a tanglefoot fly paper were cut out from tissue papers of different colours. Each piece was pressed between two fresh fly papers one of which was slightly warmed after pressing. The warmed fly paper was slowly taken off, leaving the piece of the coloured tissue paper stuck to the other fly paper. The exposed surface of the coloured paper, prepared in this manner, was as effective in catching flies as the tanglefoot fly paper itself.

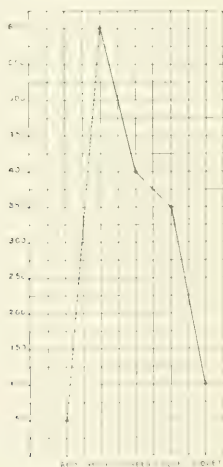
Different coloured papers (red, yellow, green, blue and violet), prepared by the method described above, were exposed on a table near a window. Light was uniform on all the papers which were kept exposed

for more than seventy-two hours at a time. The following table gives the results of these experiments:

TABLE IV.

Colour.	M. DIVARICATA.		M. PROMISCA.	
	Females.	Males.	Females.	Males.
Red .. ..	18	9	1	1
Yellow .. .	329	233	24	24
Green .. .	230	171	11	3
Blue .. .	205	139	10	3
Violet .. .	12	38	11	9
Percentage ..	58%	42%	59%	41%
Percentage present in nature at the time.	63%	37%	76%	24%

CHART II.



Number of flies attracted.

From the above table as well as from Chart II it will be seen that yellow has still the greatest attraction for flies even by day and red and violet the least. Green and blue are intermediate.

There does not seem, from the figures quoted above, any reason to conclude that females show any marked difference of preference for colours from males.



CONCLUSIONS.

1. Houseflies do respond to different colours. Whether this is a true colour preference or dependent on relative intensity of the colours has not yet been investigated.

2. Yellow has the greatest attraction, red and violet the least; blue, green and orange are intermediate.

3. There is no evidence forthcoming to show that females and males are differentiated in any marked degree in their response to various colours.

4. Response to colours by houseflies is identical by day and by night.

Finally, I have to express my grateful thanks to Lt.-Col. Buchanan, I.M.S., Civil Surgeon of Nagpur, for giving me ample accommodation for work.

# BIONOMICS OF HOUSEFLIES.

## III.

### A PRELIMINARY NOTE ON ATTRACTION OF HOUSEFLIES TO CERTAIN FERMENTING AND PUTREFYING SUBSTANCES.

BY

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AND

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[Received for publication, November 5, 1919.]

VARIOUS SUBSTANCES have been tried by different workers either to attract insects or to repel them. Oil of citronella, for instance, attracts small fruit flies; while some of the culecifuges repel mosquitoes. In the same way a substance may be found which will attract or repel houseflies.

Richardson<sup>1</sup> has used pure chemical substances, such as ammonia, acetic acid, alcohol, etc., in various percentages to attract flies. Morrill<sup>2</sup> has tried, on the other hand, certain decaying substances, such as meat, fish, etc., without any reference to the chemical changes which those substances were undergoing when they were exposed for the purpose of the experiments.

In this paper an attempt has been made to combine the experiments of Morrill with those of Richardson. Certain substances were allowed to ferment or putrefy and tested chemically from day to day, while they were attracting flies. It was observed that houseflies began to be attracted to a particular substance after it had undergone a certain degree of chemical change: the number of flies reached its maximum at a certain

stage of fermentation or putrefaction after which it began to fall, till at last that substance had no attraction for these insects.

The substances chosen for these experiments roughly fall under two main heads according as they contain large amounts of fermentable or putrescible components. The substances used were:—Rice, wheat, pulses (gram), egg, meat and fish.

Each substance was covered over with tangle foot fly papers which were perforated in the middle and was allowed to undergo spontaneous fermentation or putrefaction. The number of flies caught on these papers was noted from day to day till the substance had no attraction for them.

1. *Rice*.—Rice was made up with water and allowed to undergo fermentation.

TABLE I.

Day of exposure.	Reaction to litmus paper.	Chemical substances present.	No. of HOUSE-FLIES PRESENT.	
			M. divaricata.	M. promisca.
1 Day.	Neutral to acid.	Gas.	..	..
2	Acid	Gas, no ammonia	..	..
3	Acid	Slight alcohol, sour smell.	..	..
4	Acid	Alcohol, acetic acid	..	..
5	Acid	Alcohol, acetic acid	..	..
6	Acid	Acetic acid (strong)	..	..
7	An excess of ammonia was passed into the mixture.			
8, 9	Alkaline	Ammonia, acetic acid, Alcohol.	27	..
10	Acid	Same as above	22	1
11, 12	Acid	Same as above but slight ammonia.	34	1
13, 14	Acid	Plenty of acetic acid and alcohol, slight ammonia.	7	..
15, 16	Acid	Same as above	..	..

NOTE. Tests used for the following substances.

1. Acetic acid .. (1) Sulphuric acid (pungent smell), (2) ferrie chloride (deep red colour).
2. Alcohol .. Iodine and potassium hydroxide (smell of iodo-form was given).
3. Ammonia .. Boiling with KOH (vapour of ammonia).
4. Sugar .. Copper sulphate and KOH (brown precipitate).

It will be seen from the above table that the rice-water mixture remained acid in reaction throughout the experiment. It did not attract flies at all, though the mixture came to contain plenty of acetic acid and alcohol. Houseflies began to be attracted only after an excess of ammonia was passed through the mixture on the 7th day. The number of flies attracted continued to increase as long as there was a smell of ammonia.

## 2. *Wheat.*

TABLE II.

Day of exposure.	Reaction to litmus paper.	Chemical substances contained.	No. of HOUSEFLIES.	
			M. divaricata.	M. promisca.
1	Neutral	....	..	..
2	Acid	Gas, sugar, no acetic acid	..	..
3	Acid	Alcohol, acetic acid, sugar, no ammonia.	16	..
4, 5	Acid	Sugar decreasing, ammonia, acetic acid, alcohol.	10	1
6, 7	Acid	Same as above ..	10	..
8, 9	Acid	Ammonia stronger, acetic acid weak ? Alcohol	25	..
10	Acid	Ammonia weak ? Slight alcohol, acetic acid.	7	..
11	Acid	Same as above ..	..	..

It will be seen from the above table that the mixture of wheat and water was acid in reaction throughout the process of fermentation. It began to attract flies of its own accord on the third day when alcohol, acetic acid and sugar were present. The mixture gave reaction for ammonia from the 4th day onward. The number of flies reached its maximum on the 8th day after which the number suddenly fell. A day or two later the mixture had no attraction for flies.

It will be seen that there is a great difference between the rice and the wheat mixtures as far as attraction for flies is concerned. In one case ammonia had to be added to the mixture before flies could be attracted to it ; while in the other, flies were attracted without addition of any strong smelling substance.

3. Pulses.

TABLE III.

Day of exposure.	Reaction to litmus paper.	Chemical substances present.	No. of HOUSEFLIES.		
			M. divaricata.	M. promisca.	
1	Neutral	.....	No flies.		
2	Acid	Gas, ammonia, alcohol, acetic acid.	9	..	
3, 4	Acid	Same as above ..	19	2	
5	Acid	No gas, ammonia, acetic acid, alcohol.	19	..	
6, 7	Acid	Same as above ..	22	..	
8	Slightly alkaline	Ammonia, alcohol, acetic acid.	28	..	
9, 10, 11	Alkaline	Ammonia less ? Acetic acid, alcohol.	17	1	
12	Alkaline	Ammonia same, plenty of acetic acid, alcohol.	46	..	
13	Alkaline	Same as above ..	52	3	
14	Alkaline	Acetic acid less ? Trace of alcohol, ammonia.	69	..	
15	Alkaline	Acetic acid ? Ammonia less ? Alcohol.	37	1	
16	Alkaline	More alcohol ? More acetic acid ? Ammonia.	71	6	
17	Alkaline	Ammonia increased, acetic acid, and alcohol less.	Plenty of flies hovering over a cage in which the mixture was kept.		
18	Alkaline	Same as above ..	171	4	
19	Alkaline	Same as above ..	236	6	
20	Alkaline	Ammonia more, acetic acid and alcohol increased.	103	..	
21	Alkaline	No ammonia less ? Plenty of acetic acid and alcohol.	211	5	
22	Alkaline	Less ammonia ? Plenty of acetic acid and alcohol.	217	8	
23	The experiment suddenly came to an end owing to an accident.				

The above table will show that the mixture of the pulses and water was acid during the first few days and became alkaline thereafter. So long as the mixture was acid in reaction, few flies were attracted, but a larger number appeared on it when the mixture became distinctly alkaline. The number of flies was slowly rising and reached its maximum on the 18th to 19th day.

The greater number of flies attracted may be due to the presence of putrescible components in the pulses and hence to the production of strongly smelling and volatile products connected with that putrefaction

4. *Egg*.—The constituents of an egg differ totally from those of the substances described above, in as much as these are wholly putrescible and not a mixture of fermentable and putrescible components.

(a) Pure yellow (yolk).

TABLE IV.

Day of exposure.	Reaction to litmus paper.	Chemical substances present.	No. of houseflies.	
			M. divaricata.	M. promisca.
1	Neutral or slightly alkaline.	Slight ammonia ?	No flies.	
2, 3	Acid	Bad smell, ammonia	464	7
4	Acid more	Plenty of ammonia, acetic acid, alcohol.	199	11
5	Highly acid	Same as above	235	9
6	Acid	Same as above but slight alcohol.	170	8
7	Acid	Same as above	232	5
8	Less acid	Ammonia ? Acetic acid, alcohol.	90	6
9, 10	Acid	Ammonia ? Other substances same as above.	..	..

The mixture of the pure yellow with water was acid in reaction all the time of its putrefaction. It attracted the greatest number of flies on the 2nd to 3rd day. The number of flies attracted on other days was always very considerable, though it never reached a similar number to that of the 2nd to 3rd day.

(b) Egg-white (egg albumin).

TABLE V.

Day of exposure.	Reaction to litmus paper.	Chemical substances present.	No. of house flies.	
			M. divaricata.	M. promisca.
1	Alkaline	Slight ammonia on long heating with KOH.	No flies.	
2, 3	Alkaline	Slight ammonia	43	2
4	Alkaline	Pleasant smell, slight ammonia, trace of alcohol.	44	..
5	Alkaline strongly	More ammonia, no acetic acid, no alcohol.	48	2
6	Alkaline	Less ammonia ? Slight acetic acid, alcohol.	54	2
7	Alkaline	Same as above	87	8
8	Alkaline	Ammonia ? Acetic acid, slight alcohol.	19	..
9	Alkaline	More of acetic acid, alcohol.	Scarcely any flies.	

It will be seen from the above table that the mixture of the white and water was alkaline throughout the whole time. It attracted the maximum number of flies on the 7th day and that number, too, was much smaller than what was obtained in the case of the yellow.

Thus the egg-white mixture shows a great contrast to that of the yellow, as far as the number of flies attracted is concerned. This paucity of flies in the case of the white may be due to the absence of strong smelling volatile substances which may, very probably, be the products of putrefaction of the yellow.

The following analyses of egg-white and yellow of egg respectively given by Simon in his text-book of Physiological Chemistry may throw some light on the statements given above :—

Analysis of albumin (egg-white).

Water	..	..	..	..	80.00—86.68
Solids	..	..	..	..	13.32—20.00
Albumins	..	..	..	..	11.50—12.27
Extractives	..	..	..	..	0.38—0.77
Fats and soaps	..	..	..	..	Traces.
Mineral salts	..	..	..	..	0.30—0.66
Lecithin and cholesterin	..	..	..	..	Traces.

Analysis of yolk.

Water	..	..	..	..	47.19—51.49
Solids	..	..	..	..	48.51—42.81
Fats (olein, palmitin and stearin)	..	..	..	..	21.30—22.84
Vitellin and other albumins	..	..	..	..	15.63—15.76
Lecithin	..	..	..	..	8.43—10.72
Cholesterin	..	..	..	..	0.44—1.75
Cerebrin	..	..	..	..	0.30
Mineral salts	..	..	..	..	3.33—0.36
Colouring matter and glucose	..	..	..	..	0.553

These analyses suggest that the disintegration of such constituents as lecithin, cholesterin and cerebrin may be the cause of preference shown by flies for yolk of egg over white.

5. *Meat and fish.*



TABLE VI.

*Fish.*

Day of exposure.	Reaction to litmus paper.	Sarcophaga.	Calliphora.	Pycnosoma.	NO. OF HOUSEFLIES.	
					M. divaricata.	M. promisca.
1	Neutral to alkaline.	..	..	..	Scarcely any.	
2	Alkaline	15	1	52	1011	194
3	Alkaline	11 Plenty of larvæ.	..	117	927	200
4	Alkaline	3 Plenty of larvæ.	..	17	767	47
5	Alkaline	Plenty of larvæ.	..	2	692	23
6	Only bones were left with plenty of Sarcophaga larvæ.					

TABLE VII.

*Meat.*

Day of exposure.	Reaction to litmus paper.	Sarcophaga.	Calliphora.	Pycnosoma.	NO. OF HOUSEFLIES.	
					M. divaricata.	M. promisca.
1	Acid to neutral.	..	..	..	Scarcely any.	
2	Alkaline	..	..	35	116	2
3	Alkaline	10	..	285	605	186
4	Alkaline	13 Plenty of larvæ.	..	69	705	84
5	Alkaline	17 Plenty of larvæ.	..	85	429	24
6	Nothing was left of the solution which had been eaten by Sarcophaga larvæ.					

It will be seen from the above tables that the reactions in both the cases were alkaline except on the first day when it was acid or neutral. The flies were not at all attracted on this day but they appeared in larger numbers on the following days when the mixtures became alkaline.

The highest number of flies were attracted on the 2nd day in the case of fish, while in that of meat on the 3rd day.

Side by side with these substances an egg was allowed to putrefy and the number of flies attracted was counted.

TABLE VIII.

*Egg.*

Day of exposure.	Reaction to litmus paper.	Sarcophaga.	Calliphora.	Pycnosoma.	No. of houseflies.	
					M. divaricata.	M. promisca.
1	Alkaline to neutral.	..	..	..	..	..
2	Acid	..	..	..	239	19
3	Acid	Plenty of larvæ.	..	11	673	74
4	Acid	18 Plenty of larvæ.	..	76	914	88
5	Acid ?	Swarming with larvæ.	..	89	1026	52
6	The mixture	was simply	swarming with Sarcophaga larvæ and was nearly dried.			

From the above table it will be seen that the maximum number of flies attracted is little less than that by fish and more than that by meat.

It seems that the putrefaction products of fish and egg may be more or less similar to each other and different from those of meat. Both fish and eggs contain organic compounds of phosphorus which are not found in meat. This may account for the greater attractiveness of fish and egg for flies as compared with that of meat or it may be due to some other type products of decomposition common to both.

## CONCLUSIONS.

1. Some strong smelling substances connected with putrefaction such as ammonia, sulphuretted hydrogen, smelling organic compounds of phosphorus, etc., may be necessary to attract flies before they approach what is otherwise a satisfactory food.

2. Alkalinity or acidity of a fermenting or putrefying mixture has nothing to do with attraction of flies.

3. In none of the substances have houseflies laid eggs.

## REFERENCES.

- (1) *Journal of Economic Entomology*, Vol. X.
- (2) *Journal of Economic Entomology*, Vol. VII.

## ON THE OCCURRENCE OF COLEOPTERA IN THE HUMAN INTESTINE.

BY

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[Received for publication, September 20, 1919.]

THE existence of a pathological condition caused by the presence of beetles in the intestine was first brought to my notice by Dr. H. Ludovici of the Ceylon Medical Service, who informed me that he had seen such when District Medical Officer, Matara. It was some time before I was able to secure specimens, but at last I succeeded in obtaining such through the kindness of Dr. F. Pandiasekera, Ceylon Medical Service, at that time D.M.O., Matale, who at the same time gave the following details regarding the case:—The specimens were passed, *living*, in the faeces of a Sinhalese boy of about 4 years of age, suffering from slight temperature, cough, pain in abdomen and soft stools, at Matale Civil Hospital, following on the administration of an anthelmintic. The beetles were in the imaginal state, which agrees with Dr. Ludovici's description, that the beetles, on being passed, clear themselves from the stool, and fly off.

The specimens were submitted to the Imperial Entomologist, who informs me that they are *Orthopagus bifasciatus*, Fb., being *Scarabeida*, section *Coprini*.

From inquiries made the attacks appear to be confined to children of from 3 to 8 years of age—(though there is one report of a woman of 35 who is said to have had several attacks).—and to occur only in the low country of the Island, being reported from Matara, Southern Province (15 ft.); Anuradhapura, North-Central Province (278 ft.); Kurunegala, North-Western Province (381 ft.); Godakawela, Sabaragamuwa Province



♀ from faeces of boy in Matale Hospital, Ceylon, 30th April, 1919.

*Onthophagus bifasciatus*, Fr.

♀ on dead locusts, Bombay, 20th June, 1904.

♂ on dead locusts, Bombay, 20th June, 1904.

(The figures are considerably enlarged, the smaller figures showing the natural sizes.)



(circa 700 ft) ; and Matale, Central Province (1,208 ft.). It occurs both in Sinhalese and Tamil children.

Though seldom or never serious, the disease is sufficiently common to have a definite vernacular name, being in Sinhalese "*Kurumini Māndāmā*" (lit. "beetle-disease").

My Estate Apothecary, formerly in Government service, informs me that he has seen the imagines in the lower intestine at a *post mortem* at the Civil Hospital, Anuradhapura, where the cause of death was suspected to be anchylostomiasis. Three beetles were found, of which two were dead.

The method of invasion is obscure. The Imperial Entomologist informs me that he has this species from Poona, Bombay, on dead locust; Kurnul, and Coimbatore, on dead snake. Lefroy (*Indian Insect Life*, p. 249-250) states that the genus comprises feeders on dung and on dead insects, and has been reared from larvæ in dung balls. The particular species concerned in this case is not mentioned.

It is probable that whilst in the intestine the larvæ are fæcal feeders, but how, and at what stage, they get there, and how they remain unpassed during larval and pupal stages, is unknown. It might be mentioned that the specimens in the Matale case were all females, and that the male has a long cephalic spine, lacking in the female.

Though native children playing about untended will put most things edible into their mouths, dung, dead insects and snakes are not likely to be amongst them, and it occurs to me that possibly infection takes place through ova or young larvæ in one or other of the semi-decayed and exceedingly odoriferous forms of dried fish that form a regular article of diet with both the Sinhalese villager and the Tamil estate coolie. This, however, is merely a suggestion.

# THE CORRELATION BETWEEN THE CHEMICAL COMPOSITION OF ANTHELMINTICS AND THEIR THERAPEUTIC VALUES IN CONNECTION WITH THE HOOKWORM INQUIRY IN THE MADRAS PRESIDENCY.

BY

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## II. OLEUM CHENOPODII.

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### HISTORY.

AMERICAN wormseed oil, official in the United States Pharmacopœia as *Oleum chenopodii*, has long been in vogue in that country as a household remedy for worms, particularly for Ascarids. Injudicious use of



the drug has, however, not unfrequently resulted in poisoning as may be gathered from cases reported in medical journals and in newspapers.

The first death recorded as being due to oil of chenopodium is that of a six years old child, in 1851. It occurred 36 hours after the administration of a dose—presumably less than one ounce—of ‘wormseed oil’ obtained from an itinerant quack, and to be given in doses of 15 drops until worms were expelled<sup>1</sup>.

In 1875, Paramore<sup>3</sup> reported the deaths of three children, aged 6, 3, and 1, respectively. They belonged to the same family and were treated at the same time for worms. The eldest received eight drops three times daily for two days, and eight drops on the third day, and died in four days. The second child was given six drops three times daily for two days, and six drops on the third day; it died in five days. The infant died in eight days after having received four drops, three times daily for two days, and four drops on the third day. The symptoms observed were the same for the three children: drowsiness, thirst, fever, nausea and vomiting, coma. In no case had the oil been followed by a purge.

Three years later, Brown<sup>4</sup> cited the case of a patient, aged 31, who died five days after taking  $1\frac{1}{2}$  ounces of chenopodium and 30 drops of turpentine at a single dose.

The *New York Tribune*, June 15, 1880, reported the death of an adult who was found in coma after taking two doses of 30 grains each of oil three hours apart. And, about the same time, Gable<sup>5</sup> mentioned the case of a patient, aged 63, who succumbed to the administration of an ounce of oil in divided doses in one day. Pole<sup>7</sup> and North<sup>6</sup> also chronicle three cases of poisoning with oil of chenopodium, which, however, did not prove fatal.

That period in the history of chenopodium oil as an anthelmintic ends with two more deaths:—that of a two years old child (1897)<sup>9</sup> who received  $\frac{1}{2}$  drachm of oil two days after the administration of 10 drops which had been ineffectual, and that of a mentally deficient epileptic woman, aged 61, who administered to herself one tablespoonful of the drug (1903)<sup>10</sup>.

Having heard from two German doctors in Brazil that oil of chenopodium had long been in use there as an anthelmintic, Baumler,<sup>8</sup> of Fribourg, introduced the drug in Europe and treated a case of ankylostomiasis with it, in 1881. The treatment turned out a failure.

In 1905 Calmette and Breton<sup>11</sup> reported that the oil had been tried in ankylostomiasis, but had proved inefficient.

Bruning<sup>12</sup> began using oil of chenopodium successfully about 1909, and recommended it to Schüffner and Vervoort<sup>17</sup> who, in 1913, published the results they had obtained at the Senembah Maatschappij Hospital, Deli, Sumatra. These show the anthelmintic superiority of the oil over thymol, betanaphthol, and eucalyptus oil. Schüffner and Vervoort administered 3 c.c. oil in three hourly doses of 16 drops followed by castor oil (17 grammes) and chloroform (3 grammes) and found that 91 per cent of the worms were expelled. In the absence of chloroform the percentage of worms removed fell to 84.

At the meeting of the British Medical Association held at Aberdeen in 1914, Macallan<sup>23</sup>, of Cairo, declared that oil of chenopodium given in doses of 45 minims on sugar and followed by castor oil and chloroform was inefficient. In the course of the discussion which followed, Sandwith, of London, suggested that perhaps the oil supplied to Egypt was not of the same quality as that employed in the Malay States.

Motter (1914)<sup>19</sup> urged the wider administration of chenopodium oil in America with the definite intention of pushing the use of a 'truly American remedy.' Much urging was not required, for, by that time, oil of chenopodium had become a favourite remedy for hookworms and was most highly recommended by Guthrie, Levy,<sup>18</sup> Fort,<sup>22</sup> Bishop and Brosius.<sup>21</sup> The mode of treatment generally recommended was as follows:—*1st day*: 1 oz. Epsom salts in the evening; liquid diet begun;—*2nd day*: liquid diet; 1 oz. Epsom salts in the evening;—*3rd day*: three doses of 16 minims or drops of chenopodium oil, in capsule or on a teaspoonful of granulated sugar, at intervals of two hours. Two hours after the last dose of chenopodium 1 oz. castor oil was administered together with 45 or 50 minims chloroform; soft supper. The treatment was repeated at weekly intervals until ova could no longer be found in the feces. The addition of chloroform to the castor oil was strongly advised, on the plea that 'it appears to assist in the narcotization of the worms in their passage through the intestinal tract.' The conclusions generally admitted were the following:—(1) Chenopodium oil is an efficient vernifuge in the treatment of uncinariasis; (2) the method of administration is simple; the drug is not unpleasant to take, its ingestion is followed by no disagreeable symptoms; (3) chenopodium oil can be given at shorter intervals than other anthelmintics and a cure can thereby be more quickly established, which gives it a

greater economic value; (4) it is nontoxic in therapeutic doses. In this connection Bishop and Brosius deserve being quoted: 'Believing in the nontoxicity of the drug, we have not hesitated administering it to all patients regardless of other conditions, with the exceptions of those suffering from hyperpyrexia and acute inflammation of the gastrointestinal tract. Accordingly, during the course of treatments, we have had some deaths all of which went to necropsy and, as will be seen, in none of these, despite careful investigation, were there any evidences of chenopodium poisoning being a factor in the cause of death.'

During 1915<sup>31</sup> a number of experiments were made with chenopodium oil. In Guatemala more than 9,000 persons were treated with this drug and particularly favourable results were obtained. Among these 9,000 persons, only two cases of untoward symptoms were observed, both in children, in the form of temporary deafness, lasting in one instance two weeks and in another four weeks. The standard adult dose was 30 drops administered at one time, or divided into two parts and given one or two hours apart. There was no noticeable difference in results by either method. The drug was not found very trying on the patient, permitting a subsequent treatment within four days.

In Nicaragua and Costa Rica<sup>31</sup> excellent results were also reported with the use of chenopodium, and no untoward symptoms observed.

In Trinidad,<sup>31</sup> however, a series of experiments with oil of chenopodium gave less satisfactory results and led Washburn to conclude that there was nothing to justify adopting this drug in place of thymol as the standard remedy for uncinariasis. Of 342 selected cases, 138 were treated by the Keith method, in which ten minims of chenopodium are given daily for three days, followed by a tablespoonful of castor oil two hours after the last dose has been taken; and the other 204 cases received the full dose on one day, followed in two hours by a large dose of castor oil. In some cases magnesium sulphate was used instead of castor oil without any appreciable difference in the result. In all of the cases treated with oil of chenopodium in Trinidad, unpleasant symptoms such as nausea, vomiting, weakness, dizziness, etc., were much more marked than with thymol, and the continued use of chenopodium in a district alarmed the people and made them afraid to take further treatment of any kind.

About the same time, Tijssen<sup>27</sup> found that oil of chenopodium would not, in any case, procure the expulsion of more than 60 per cent of

the worms present, as against the 91 per cent recorded by Schüffner and Vervoort.

The reports for the year 1916,<sup>34</sup> as to the value of chenopodium in the treatment of hookworm infection, are conflicting in character;—the result, perhaps, of differences in the strength and potency of the drug, in the laboratory technique employed, or in the methods of administration.

During the last quarter of the year, chenopodium oil mixed with eucalyptus oil (three parts chenopodium and one part eucalyptus) was given to the recruits of the Nicaraguan army,<sup>34</sup> and the results seemed to indicate that the addition of oil of eucalyptus increased the efficiency of the drug.

Moillet and Carrero,<sup>28</sup> in Mexico, had already had recourse to a mixture containing oil of eucalyptus, oil of chenopodium, chloroform and castor oil, but the results obtained from this mode of treatment were not recorded.

During 1917, Molloy<sup>38</sup> tried the same mixture (chloroform 45 drops, chenopodium and eucalyptus oil each 20 drops, and castor oil one and one half ounces), but on account of the difficulty of dispensing, it was not used to any considerable extent in field work.

By this time the literature emphatically insists on the injurious action of the drug on the host animal. The Rockefeller Commission<sup>34</sup> note this undesirable effect in the following terms:—‘Alarming symptoms, and sometimes deaths, have been reported in the Southern States, the West Indian Colonies, Panama, Nicaragua, Ceylon, and Egypt following the administration of the drug in accordance with accepted methods of treatment, and in nearly every instance in less than the maximum dose. Extreme caution in the use of the drug is therefore indicated until its proper method of preparation has been learned, its chemical composition and stability standardised and a safe dosage and method of administration established.’ And Snodgrass<sup>30</sup> declares that ‘even the smaller doses now advised for adults under 50, namely, twelve minims repeated hourly for three doses, should only be given under the directions of a medical man, since dangerous symptoms may otherwise occur.’

The Rockefeller Commission for 1917<sup>40</sup> admit that in Ceylon, Panama, Dutch Guiana, Brazil, and a number of other countries, alarming symptoms, or deaths, have sometimes followed the administration of chenopodium. They conclude that the maximum doses of chenopodium recommended in the literature are unsafe, and they advise 1.5 mls. of

chenopodium, divided into three doses of 0.5 mil. each, as the standard treatment for hookworm disease.

Two deaths in rapid succession following the administration of chenopodium oil made it necessary to abandon its use in the Dickoya Area, Ceylon (1917).<sup>11</sup> It was however continued in the Bogawantalawa, Norwood, and Matale areas. During the latter part of 1917, Winsor, in the Matale area, began a series of experiments with the object of combining both purgative and anthelmintic into a single treatment. For this purpose he used a combination of croton oil and chenopodium in the proportion of 120 minims of the former to 10 ounces of the latter. Half of the dose of croton-chenopodium oil was administered at 7 A.M., and the other half an hour later. From one and a half to two hours after the second dose, a small amount of castor oil was given. No evening purge was given prior to the administration of the anthelmintic. For the first treatment the maximum adult dose of chenopodium recommended was 32 minims, and for children, between five and ten years of age, one minim for each year of age; heavier dosages appeared to entail considerable complaint from the patients and too great risk of serious untoward results. The advantages of the croton-chenopodium oil method over the use of chenopodium alone are summarised by Winsor as follows:—(1) Croton oil is a purge which is very reliable in its action. This is of special significance in the low country, where it requires enormous doses of salts to produce purgation. The administration of croton-chenopodium oil is not accompanied by griping, probably on account of the carminative action of chenopodium; (2) the treatment is not nearly so exhausting as the double purge method formerly employed, and therefore meets with less opposition on the part of the coolies; (3) the toxic effects of chenopodium are not so noticeable as when a purge is given the night before; (4) a larger percentage of round worms are expelled than when an equal dose of chenopodium is given by the usual method; (5) a slightly higher percentage of cures results from treatment with croton-chenopodium oil than from treatment with a corresponding amount of chenopodium given in the usual way.

In October 1917, Coomarasamy and De Costa<sup>32</sup> reported four deaths which occurred in the Civil Hospital at Matale, Ceylon, following upon the ordinary routine chenopodioid treatment. The authors note that: (1) all the four patients were under seven years of age; (2) the symptoms came on within twenty-four hours of the administration of the drug; (3) in two of the cases deaths supervened within forty-eight

hours after treatment. And they conclude that 'this valuable drug may prove a dangerous one when administered injudiciously, and especially if the directions for use are not strictly adhered to.'

In February 1918, Darling, Barber and Hacker<sup>35</sup> add two more cases to the long catalogue of deaths due to the administration of oil of chenopodium. 'Both deaths occurred within ten days of each other at a time when it seemed necessary to expedite the work by giving two treatments within a week.' As the result of an exhaustive study of the chenopodioid treatment of hookworm infection the authors fix the maximum dose of oil at 3 c.c. and recommend half that amount (0.5 c.c. three times, or 1.5 c.c.) as a routine treatment. They are of opinion that castor oil as a purge is responsible for numerous cases of dizziness and deafness. They find fault with the manufactured soft capsules and recommend the use of freshly filled, hard gelatin capsules. They, moreover, show that the preliminary purge may be omitted under certain circumstances without lowering the efficacy of the drug which, however, may be greatly impaired by carrying the starvation of the patient beyond certain limits. The following diet is, therefore, advised: rice gruel in the afternoon previous to treatment, but milk on the morning of treatment. Because of the cumulative action of the drug at least a week should elapse between two treatments.

Meanwhile (1918)<sup>32</sup> the officers in charge of the Ankylostomiasis campaign in Ceylon have adopted a dosage table where the maximum dose figures as 24-28 minims, in two portions, for adults from 21 to 50 years of age. The evening before treatment only a light meal of rice gruel is allowed. The initial purge is omitted in field work, and a drink of milk and water or hot rice konjee is allowed before the drug is taken. Effective post-treatment purgation is considered essential and a second purgative is given whenever the post-treatment purgative fails to act freely and promptly (within two hours).

Darling, Barber and Hacker do not call the reader's attention to a lowering of efficiency consequent upon the addition of liquid petrolatum to the oil, as recorded in one of their tables. We think this finding deserves mention, for later in the course of the same year Hall<sup>37</sup> and his collaborators showed that the anthelmintic effect of the drug is seriously impaired by combining it with liquid petrolatum.

Following the Sumatra method, Wrench<sup>34</sup> treated his patients after their midday meal. They were given a capsule containing 1 c.c. of chenopodium oil at 1 P.M., 2 P.M., and 3 P.M., followed by 6 drachms of



castor oil at 4-30 P.M. Symptoms of poisoning by the drug, such as marked dizziness or vomiting after a dose, precluded a further capsule being given. Wrench thinks it is important to emphasize the fact that chenopodium oil is a dangerous drug.

And the last note for 1918 is one of warning from the wards of Santo Tomas Hospital, Panama:<sup>39</sup> 'The oil should not be administered unless there be ample facilities for studying the cases before and after administration. This should include a careful determination of the percentage of hæmoglobin. Chenopodium oil should not be administered to a patient suffering from a high grade of anæmia, nor should the treatment be repeated within ten days.'

In March 1919, Knowlton<sup>43</sup> shows that hard (soluble) capsules are to be preferred to soft gelatin proprietary capsules.

#### COMPOSITION AND PROPERTIES.

American wormseed oil is distilled from *Chenopodium ambrosioides*, var. *anthelmintica*.

Of the seven species of *Chenopodium* possessed by India only two—*C. ambrosioides* and *C. botrys*—have been credited with anthelmintic properties.

On April 5, 1918, we received from the Surgeon General, Madras, six sun-dried plants labelled *Chenopodium anthelminticum* grown on Koilpati Farm (Madura District). The seeds, weighing 4 grammes were steam distilled and yielded about  $\frac{1}{2}$  c.c. of a pleasant smelling colourless oil which evaporated entirely at 36°C.

Later, in June, 30 grammes of seeds from a hundred plants were submitted to steam distillation and yielded 1.3 c.c. of oil. Except for a slight difference in the smell, this oil was found to resemble the commercial oil of chenopodium and readily oxidised ferrous sulphate in saturated solution, as much as 70 per cent of the oil being reduced.

Like most of the volatile oils, oil of chenopodium is a mixture of constituents. It has no distinct boiling point and explodes with extreme violence when heated above 100°C. at atmospheric pressure. The colour is found to vary with different samples from golden yellow to pale straw yellow. The smell is characteristic, resembling turpentine in the light coloured lots. The taste is acrid, leaving a sensation of burning in the mouth.



The physical variations of the oil are recorded in Table I.

TABLE I.  
*Physical Examination of Various Samples of Chenopodium Oil.*

			Specific Gravity 25°	Specific Rotation (α) <sub>D</sub>	Refractive Index n <sub>D</sub>	Solubility in 70 per cent alcohol.		
Schammel & Co. (1908)	Oil from seed	..	0.9768	-4.5°	1.4785	1 : 4		
			15°	?	20°			
	Steam distilled sample	1..	0.9691	-5.4°	1.4726	1 : 3		
Nelson (1911)	"	"	2..	0.9700	-6.2°	1.4723	1 : 3	
	"	"	3..	0.9550	-8.8°	1.4726	1 : 7	
	Pot distilled sample	..	0.9584	-6.3°	1.4725	1 : 6		
	Aged oil	..	0.9694	-0.35°	1.4780	1 : 3		
				20°	20°			
Hamilton (1918)	Original oil 1	..	..	-6.2°	..	..		
	"	"	2	..	0.9570	-6.0°	..	
				?	?			
	Oil	II	September	1917	0.9681	-4.0°	1.4712	1 : 8
					29°	29°		
	"	"	March	1919	0.9888	-2.5°	1.4773	1 : 3
	Oil	III	September	1917	0.9658	-4.5°	1.4716	1 : 4
					"	"		
	"	"	March	1919	0.9684	4.0°	1.4730	1 : 6
					"	"		
Campbell Muller (1917-1919)	Oil	IV	April	1918	0.9599	1.6°	1.4720	1 : 6
					32	32		
	"	"	July	..	0.9618	-5.0°	1.4730	1 : 7
					31°	31		
	"	"	September	..	0.9717	-5.1°	1.4717	1 : 7
					29°	"		
	"	"	March	1919	0.9721	-5.1°	1.4717	1 : 7
	"	"	August	..	0.9692	-5.2°	1.4717	1 : 6
					31	..		

Vanderkleed<sup>6</sup> has noted the finding, in a market sample of chenopodium oil, of adulteration with 44.3 per cent of an odourless fixed oil.

The first recorded chemical examination of oil of chenopodium is that of Garrigues,<sup>2</sup> in 1854, who reported the presence of two constituents, one of which he described as a hydrocarbon boiling at 176 C. and the other as a liquid of the formula C<sub>10</sub>H<sub>16</sub>O.

Kremers<sup>13</sup> (1907) in attempting to distil the oil at atmospheric pressure found that a decomposition of extreme violence took place when the oil was heated to boiling temperature.

In 1908, Schimmel & Co.<sup>14, 15</sup> reported an extensive investigation of the oil. By fractionating at reduced pressure they separated a repulsive smelling yellow oily liquid which constituted the major portion of the oil, and to which they gave the name 'ascaridol.' On analysis ascaridol afforded the formula  $C_{10}H_{16}O$ , but its chemical nature was not determined. Toward reagents which show alcoholic, aldehydic, ketonic, or phenolic characters, the substance was found to be quite indifferent. On heating to  $150^{\circ}C$ . a molecular rearrangement apparently took place, and, on combination, the product of conversion gave values which agreed with the formula  $C_{10}H_{16}O_2$ . Ascaridol reacted violently with concentrated formic acid, or concentrated sulphuric acid giving cymene; zinc dust and acetic acid gave cymene and a ketone, probably carvenone. The lighter boiling fraction of the oil, about 22 per cent, was found to consist mainly of cymene mixed with small quantities of a terpene, probably sylvestrene. Camphor was identified in the fraction coming over before the ascaridol. The peculiar chemical as well as medicinal properties of oil of chenopodium seem to be due to ascaridol, which may be found in proportions varying from 45 to 65 per cent in different samples.

In October 1910, Nelson<sup>16</sup> collected authentic samples of chenopodium oil for the purpose of making a chemical examination. He thus verified the findings of Schimmel & Co. Ascaridol was separated by fractionation at 8 mm. Thus purified, it has a constant boiling point and probably constitutes at least 70 per cent of the sample. Its indifference toward reagents, which would characterize it as an aldehyde, ketone, phenol, or alcohol, taken with the fact that it rearranges to form a glycol analogous to pinene oxide, seems to warrant the conclusion that it is an unstable dioxide. Further evidence is afforded by its property of exploding when heated, and by the violence of its reaction with ferrous sulphate and other reducing agents. By reacting with ferrous sulphate the author obtained ascaridol-glycol whose monobenzoate (melting point =  $136^{\circ}$ — $137^{\circ}C$ .) and dibenzoate he also prepared. When oxidised with a neutral solution of potassium permanganate the glycol yielded a dibasic acid, ascaridic acid, which broke down into methylheptenone when heated above its melting point. This acid is probably closely related to cineolic acid.

In April 1918, Hamilton<sup>37</sup> recorded that the steadily rising temperature and equally steady distillation of oil of chenopodium gave no line of demarcation to indicate that any considerable quantity of a single constituent is present. Under a vacuum of 2 to 5 mm. of mercury distillation began at about 50° and the oil was completely distilled at 100°, except a gummy residue amounting to about 5 per cent. These results are distinctly at variance with those reported by Schimmel & Co. and by Nelson, both of whom separated from the oil a considerable portion with a distinct boiling point, the so-called ascaridol. Different fractions were found to be different in anthelmintic activity; the portion having the lowest boiling point (85°-100° at 30 mm.) and highest optical rotation ( $-13.9^\circ$  and  $-13.4^\circ$ ) being the most efficient. The author concludes that the product marketed as oil of chenopodium should be redistilled to eliminate a fraction which has less anthelmintic value and more irritant and toxic properties than the lighter fraction, and that such a procedure should add to the value and safety of the drug. It is also noted that shaking the oil with liquid petrolatum led to the formation of a small amount of a thick dark brown liquid which was found to be toxic and irritating but had no anthelmintic properties.

Our work was started in June 1917.

Measured quantities of the original oil were dissolved in 90 per cent alcohol and shaken vigorously with a saturated solution of ferrous sulphate. The mixture became quite hot, gas was evolved, and much basic ferric sulphate was precipitated. The reaction was so violent as to become, on two or three occasions, uncontrollable. The reduction of the oil was considered to be complete when gas ceased to be evolved and no rise of temperature occurred on further addition of ferrous sulphate. In this way it was found that from 60 to 70 per cent of the oil was reduced, the amount varying with different samples. The portion which had not reacted was separated, dried over calcium chloride, and tested for its physical, chemical, and anthelmintic properties.

This oil, which we shall call 'residue oil,' was found to have a relatively low specific gravity and high rotatory power. Difficultly soluble in alcohol at 70 per cent, it dissolved more readily in 80 per cent alcohol. It had the characteristic odour and taste of the original oil: the colour varying from yellow to orange yellow. The physical data are shown in Table II.

TABLE II.

*Physical Examination of 'Original Oils' and 'Residue Oils.'*

		Amount per cent.	Specific Gravity 25°	Specific Rotation ( $\alpha$ ) <sub>D</sub>	Refractive Index ( $n$ ) <sub>D</sub>	Solubility in alcohol.	
						70 per cent.	80 per. cent.
Original oil II	..	..	0.9888	-2.5° 29°	1.4773 29°	1 : 3	..
Residue oil A	..	38	0.9107	-9.6° "	1.4735 "	..	1 : 5
Original oil III	..	..	0.9658	-4.5°	1.4716	1 : 4	..
Residue oil B	..	40	0.9016	-9.5° "	1.4760 "	..	1 : 2
Original oil III	..	..	0.9684	-4.0°	1.4730	1 : 6	..
Residue oil C	..	40	0.9277	-9.0° "	1.4762 "	..	1 : 4
Original oil IV	..	..	0.9599	-4.6° 32°	1.4720 32°	1 : 6	..
Residue oil D	..	30	0.9060	-10.2° "	1.4795 "	..	1 : 2
Original oil IV	..	..	0.9721	-5.1° 29°	1.4717 31°	1 : 7	..
Residue oil E	..	34	0.9056	-11.6° "	1.4767 "	..	1 : 7
Original IV	..	..	0.9692	-5.2° 31°	1.4717	1 : 6	..
Residue oil F	..	34	0.9016	-11.6° "	1.4838 "	..	Insoluble.

Such reagents as hydrochloric acid, nitrous acid, mercuric sulphate showed the presence of a terpene. The oil was accordingly distilled under atmospheric pressure: distillation began at 170° and was complete at 295°, except for a black gummy residue amounting to about 15 per cent. Examination of the different fractions enabled us to identify *phellandrene*, *paracymene*, *terpinene* and *carvone*, among the products of distillation.

TABLE III.

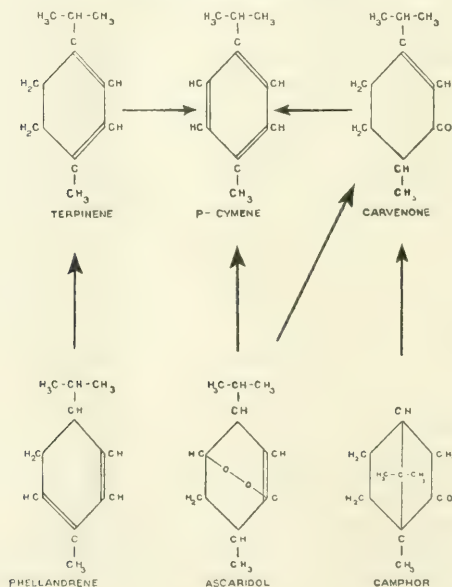
*Chemical Examination of Residue Oil D.*

Fraction.	Approximate amount per cent.	Substances identified.
170°—173°	12	Phellandrene.
173°—176°	3.5	Para-cymene.
178°—190°	1.5	Terpinene.
190°—220°	6	....
220°—250°	9	Carvenone.
250°—295°	8	....
Non-volatile residue	15	....

The residue oils had no anthelmintic value.

After separation of the 'residue oil' the aqueous solution was freed from precipitated ferric sulphate by filtration, and shaken with ether.

RELATIONS BETWEEN SOME OF THE COMPOUNDS KNOWN TO EXIST IN CHENOPODIUM OIL.



On evaporating the ether a dark viscous residuum was obtained. This was treated with water when a portion went into solution leaving behind a thick tarry substance. The aqueous solution was extracted with ether, and on evaporating there remained a yellow viscous substance with a faint smell and burning taste. This substance was benzoylated (Schotten-Baumann process) and the ester filtered off, dried, and crystallised from alcohol; (melting point =  $135^{\circ}$ — $136.5^{\circ}$ ). The product of the Schotten-Baumann reaction was, therefore, identical with Nelson's 'monobenzoate of ascaridol-glycol.'

#### *Discussion of Results.*

1. Oil of chenopodium is a mixture of constituents whose nature has not yet been fully established.

Our results confirm the existence of a peroxide, ascaridol, as reported by Schimmel & Co., and by Nelson. In addition to para-cymene, mentioned by those authors, we have identified two terpenes: phellandrene and terpinene. Whether carvenone and camphor are present in the original oil is still a matter of doubt.

2. The anthelmintic activity of the oil disappears on complete reduction with ferrous sulphate.

Schimmel & Co.'s surmise that the medicinal properties of oil of chenopodium are due to ascaridol is thus verified.

3. The percentage of ascaridol varies in different oils.

Schimmel & Co. found ascaridol in proportions varying from 45 to 65 per cent in different samples of oil. Nelson stated that it probably constituted at least 70 per cent of a sample he experimented upon. We have found the amount to be 60, 62 and 70 per cent, respectively, for three different samples.

4. Ageing may alter the physical constants of the same oil.

This may easily be gathered from our work as recorded in Table I.

5. Changes in the physical constants are associated with a variation in the percentage of ascaridol.

We see in Table II that after a period of eighteen months the proportion of ascaridol—60 per cent—was the same in an oil—Oil III—whose physical constants had but very slightly varied during that time, while the percentage had fallen from 70 to 66 in another oil—Oil IV—which showed more evident signs of physical alteration for a period of seventeen months. The study of the physiological properties

of the oil will later reveal that such an apparently small variation as 4 per cent ought not to be overlooked.

Considering the above we have thought advisable to divide the second portion of our inquiry into two sections:—

*Section A*:—Wherein all the conclusions are based upon experiments carried on with the same kind of oil—Oil IV—containing 66 per cent of ascaridol, and

*Section B*:—Wherein results arrived at with different samples of oil are compared and discussed.

## SECTION A.

### ABSORPTION AND ELIMINATION.

*Previous investigations*:—Darling, Barber and Hacker record having examined the urine of patients after treatment with chenopodium oil and found that a substance which reduces Fehling's solution is frequently present and that the cold nitric acid test gives a ring of 'oleoresin' in most cases. They conclude that absorption and excretion in the urine occur with the drug and assert that chenopodium has been detected in the urine up to 126 hours after its administration.

*Procedure adopted*:—Healthy male convicts from the Trichinopoly Central Jail were selected for the purpose of our investigation. They were given a light diet at 5 P.M. followed by a magnesium sulphate purge at 8 P.M. The next morning they were asked to urinate and were then treated with chenopodium oil dispensed on sugar. Epsom salts were given two hours after the administration of the last dose of oil.

The urine and feces discharged after treatment were separately collected for examination.

Note was taken of the colour, volume, density and acidity of the urine, which was clinically examined for albumin and indican. Measured quantities were then treated with hydrochloric acid and either distilled or examined for glycuronic compounds.

A watery fecal extract was prepared by collecting the stools in three litres of water holding 15 grains of copper sulphate in solution, and extracting for a day with frequent shaking. This was examined for 'chenopodium.'

The species, number, and the condition of the worms expelled after the chenopodiol test-treatment were noted, and the patient's total hookworm content was determined by repeating the thymol standard-treatment until cure followed.



*Experiments* :—1. A concentrated solution of ferrous sulphate proved a very sensitive test for ascaridol, as little as  $\frac{1}{4}$  minim of the original oil causing immediate oxidation and precipitation of the ferric salt.

2. It was found that copper sulphate does not react with the oil and that its presence does not prevent the immediate oxidation of ferrous sulphate.

3. A series of 50 experiments with urines, both normal and chenopodiated, showed that neither the formation of 'oleoresin' with cold nitric acid nor the colour changes with hydrochloric acid can be depended upon for the detection of 'chenopodium.'

4. 100 c.c. of chenopodiated urine were treated with 20 c.c. strong hydrochloric acid and distilled, when it was found that the first portion which passed over strongly smelt of thyme. No beading occurred, nor did oily drops form during the process of distillation, nor could any trace of ascaridol be detected in the distillate.

5. The presence of glycuronic acid was determined by the method of Nicolas. To 50 c.c. of chenopodiated urine an equal volume of strong hydrochloric acid was added and the mixture quickly heated to boiling. It was then cooled under the tap, introduced in a separating funnel, and shaken with carbon disulphide which acquired a green fluorescence in the presence of glycuronic compounds.

6. By allowing 1 c.c. of chenopodium oil to remain in intimate contact with a mass of feces for 24 hours it was found that the chenopodium smell was noticeable in the watery extract and could be made more apparent by heating after acidulating with hydrochloric acid. There was, however, no oxidation of ferrous sulphate.

*Results* :—So far we have depended entirely on experiments 4 and 5 for the study of the absorption and elimination of the constituents of oil of chenopodium, and consequently the subject has been but lightly touched upon, for in the absence of a reliable quantitative method we did not think it worth our while to accumulate figures necessarily doubtful.

1. *The Original Oil* :—

(a) 40 minims of oil on sugar were administered in three portions to twelve cases and 24 minims in one portion to four other cases. The urine was collected for the period of 24 hours which followed the administration of the first dose. Glycuronic acid was present and the thymy smell was apparent in all cases.

TABLE IV.

*Examination of urines passed during the 24 hours which followed the chenopodiol treatment.*

Case number.	Dosage.	Volume for 24 hours.	Density.	Glycuronic acid.	Thymy smell.
1	40 minims in three doses.	564 c.c.	1030	+	+
2	Ditto. ..	812 ..	1024	+	+
3	Ditto. ..	624 ..	1024	+	+
4	Ditto. ..	1122 ..	1012	±	+
5	Ditto. ..	1500 ..	1012	+	+
6	Ditto. ..	918 ..	1014	+	+
7	Ditto. ..	1258 ..	1014	+	+
8	Ditto. ..	914 ..	1020	±	+
9	Ditto. ..	800 ..	1024	+	+
10	Ditto. ..	.....	.....	±	+
11	Ditto. ..	886 c.c.	1016	±	±
12	Ditto. ..	922 ..	1016	±	±
13	24 minims in one dose ..	1036 ..	1009	+	+
14	Ditto. ..	1204 ..	1010	+	+
15	Ditto. ..	1080 ..	1008	±	+
16	Ditto. ..	1002 ..	1010	±	+

+ = present ; + + = in large amount ; ± = in small amount.

As seen from this table as well as from Table V : -

i. absorption and elimination through the kidneys occur with chenopodium oil ;

ii. there seems to be wide variation in the amount excreted from the same dose ;

iii. the elimination seems to be independent of the volume and the density of the urine.

(b) Six cases received 40 minims in three portions and four cases 24 minims in one dose. Their urine was collected for every period of 24 hours from the time of the administration of the first portion during two, three, or four consecutive days. The elimination of chenopodium takes place during the 24 hours which follow treatment with the drug, and is surely completed within 48 hours.

TABLE V.

*Time taken for complete "chenopodium" elimination.*

Case Number.	Dosage.	Time of collection.	Volume for the period.	Density.	Glycuro-nic acid.	Thymy smell.
13	24 minims in one dose ..	{ 1st day ..	1036 c.c.	1009	+	+
		{ 2nd " ..	886 "	1010	0	0
		{ 3rd " ..	1496 "	1010	0	0
14	Ditto ..	{ 1st " ..	1204 "	1010	+	+
		{ 2nd " ..	1492 "	1010	±	±
		{ 3rd " ..	1482 "	1008	0	0
15	Ditto ..	{ 1st " ..	1080 "	1008	+	+
		{ 2nd " ..	972 "	1010	0	0
16	Ditto ..	{ 1st " ..	1002 "	1010	±	+
		{ 2nd " ..	1060 "	1012	0	0
17	40 minims in three doses ..	{ 1st day ..	878 "	1021	+	+
		{ 2nd " ..	1838 "	1005	0	0
18	Ditto ..	{ 1st " ..	818 "	1018	+	+
		{ 2nd " ..	2010 "	1008	±	0
19	Ditto ..	{ 1st " ..	1086 "	1010	+	+
		{ 2nd " ..	2700 "	1010	0	0
		{ 3rd " ..	2820 "	1006	0	0
20	Ditto ..	{ 1st " ..	942 "	1016	+	+
		{ 2nd " ..	1660 "	1010	0	0
		{ 3rd " ..	1660 "	1010	0	0
21	Ditto ..	{ 1st " ..	1244 "	1010	±	+
		{ 2nd " ..	1364 "	1010	0	±
		{ 3rd " ..	1482 "	1010	0	0
		{ 4th " ..	1380 "	1008	0	0
22	Ditto ..	{ 1st " ..	950 "	1015	-	+
		{ 2nd " ..	1170 "	1010	±	+
		{ 3rd " ..	1940 "	1006	0	0
		{ 4th " ..	1556 "	1010	0	0

(c) In six cases the day urines were collected at regular intervals of four hours after the administration of the first dose, thus forming three samples. The fourth collection consisted of the 12 hours night urine. The findings do not throw any additional light on the results mentioned above.

2. *The residue oil*, in 48 minims dosage, behaved in the same way as the original oil, but the thymy smell was much stronger.

3. *Ascaridol glycol* was also found to have passed in conjugation with glycuronic acid. No smell of thyme was, however, perceived on distilling the urine with hydrochloric acid.

4. *Chenopodiated urine*:—The colour of the urine after treatment with oil of chenopodium varied from pale straw yellow to dark brown, depending mostly on the volume. Its odour was not in any way characteristic. Its reaction was acid. The presence of glycuronic compounds did not prevent decomposition.

As a rule, the density was normal, and also the volume, whenever the patient drank freely after treatment.

Indican was generally absent. 150 cases were examined for albumin and no albuminuria was found to have occurred.

5. No 'chenopodium' could be detected in the watery fecal extracts which smelled strongly of sulphuretted hydrogen and showed a deposit of free sulphur. When distilling with hydrochloric acid the smell of scatol became predominant.

*Discussion of results.*—1. The constituents of chenopodium oil are partly absorbed and eliminated through the kidneys in conjugation with glycuronic acid.

This explains why Darling, Barber and Hacker found that a substance which reduces Fehling's solution is frequently present in the urine of patients after chenopodiol treatment.

2. The elimination generally takes place during the 24 hours which follow the treatment, and never extends beyond 48 hours.

Darling, Barber and Hacker's assertion that chenopodium has been detected in the urine up to 126 hours after its administration was based on the oleoresin test which very often obtains with normal urines.

3. No chenopodium could be recovered from the feces.

This fact, taken together with the relatively rapid absorption of the drug, naturally leads us to question the necessity of the after-purge.

#### THE RATIONALE OF CHENOPODIUM OIL TREATMENT.

1. *The dosage.*—In this part of the investigation our first concern was to ascertain as definitely as possible the maximal vermifugal dose of chenopodium oil to be administered in cases of hookworm infection among adult males. Our experiments were accordingly carried out on adult and apparently healthy male prisoners whom we divided into ten batches to be treated with different dosages: 8, 16, 24, 32, 40 and 48 minims. Six of the groups were given the dose in three portions at hourly intervals, while the other four received it in one single portion. The amount of oil required for one particular group was measured out, shaken with gum acacia emulsion on the spot, and one ounce of the

mixture was dispensed to every case. The alimentary canal had previously been prepared by purgation. The test treatment was followed by a purgative administered two hours after the last portion of the oil. Epsom salts were used throughout.

60 grains thymol in hourly 20-grain doses constituted our standard treatment and was used in all subsequent treatments to ascertain the total number of worms in each case.

We fixed upon 8 minims as the starting submaximal dose with a view to follow both the possible toxic symptoms in the host and the hookworm removal with subsequent increasing dosages.

The stools passed after treatment were collected, washed, and the total hookworm content determined in the usual routine way.

TABLE VI.

*Number of hookworms removed by one "test treatment" of chenopodium oil No. IV, in three portions.*

Experiment number.	Test treatment.	Number of cases treated.	HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A "TEST TREATMENT."			
			A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.	
1	8 minims ..	19	Test treatment ..	7	389	396	21.2	64.4	62.1
			Subsequent treatments.	26	213	241			
			Total hookworms..	33	604	637			
2	16 Do. ..	20	Test treatment ..	7	210	217	38.8	65.6	64.2
			Subsequent treatments.	11	110	121			
			Total hookworms..	18	320	338			
3	24 Do. ..	19	Test treatment ..	51	594	645	78.4	78.2	78.2
			Subsequent treatments.	14	165	179			
			Total hookworms..	65	759	824			
4	32 Do. ..	18	Test treatment ..	22	268	290	81.4	83.2	83.1
			Subsequent treatments.	5	54	59			
			Total hookworms..	27	322	349			
5	40 Do. ..	18	Test treatment ..	63	981	1,044	80.7	94.1	93.1
			Subsequent treatments.	15	61	76			
			Total hookworms..	78	1,042	1,120			
6	48 Do. ..	23	Test treatment ..	73	1,040	1,113	76.8	95.3	93.7
			Subsequent treatments.	22	51	73			
			Total hookworms..	95	1,091	1,186			

TABLE VII.

*Number of hookworms removed by one "test treatment" of chenopodium oil No. IV, in one portion.*

Experiment number.	Test treatment.	Number of cases treated.	HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A "TEST TREATMENT."			
			A. duode-nale.	N. ame-ricanus.	A. duode-nale and N. ame-ricanus.	A. duode-nale.	N. ame-ricanus.	A. duode-nale and N. ame-ricanus.	
7	8 minims ..	17	Test treatment ..	23	262	285	79.3	74.4	74.8
			Subsequent treat-ments.	6	90	96			
			Total hookworms ..	29	352	381			
8	16 Do. ..	17	Test treatment ..	7	268	275	70.0	77.2	77.0
			Subsequent treat-ments.	3	79	82			
			Total hookworms..	10	347	357			
9	24 Do. ..	52	Test treatment ..	81	1,438	1,519	60.9	83.4	81.8
			Subsequent treat-ments.	53	285	338			
			Total hookworms..	134	1,723	1,857			
10	32 Do. ..	20	Test treatment ..	27	414	441	87.1	97.1	96.3
			Subsequent treat-ments.	4	12	16			
			Total hookworms..	31	426	457			

TABLE VII(a).

*Anthelmintic action of 32 minims dosage of chenopodium oil No. IV.*

Test treatment.	Number of cases treated.	HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A "TEST TREATMENT."			
		A. duode-nale.	N. ame-ricanus.	A. duode-nale and N. ame-ricanus.	A. duode-nale.	N. ame-ricanus.	A. duode-nale and N. ame-ricanus.	
In <i>three</i> portions at hourly intervals.	18	{ Test treatment ..	22	268	290	81.4	83.2	83.1
		{ Subsequent treat-ments.	5	54	59			
		{ Total hookworms..	27	322	349			
In <i>two</i> portions at an hour's interval.	11	{ Test treatment ..	61	696	757	75.3	88.7	87.5
		{ Subsequent treat-ments.	20	88	108			
		{ Total hookworms..	81	784	865			
In <i>one</i> portion ..	20	{ Test treatment ..	27	414	441	87.1	97.1	96.3
		{ Subsequent treat-ments.	4	12	16			
		{ Total hookworms..	31	426	457			

The results as figured in Tables VI, VII and VII(a) show that :—

(a) Whether the drug be administered in one portion or in three, its anthelmintic effect increases steadily with the dosage.

(b) For the same dosage the anthelmintic action of the drug is more marked in the case of the one dose treatment than in the case of the two or three doses treatment.

(c) 32 minims in one portion or 48 minims in three portions may be considered as the optimum dosage in case of hookworm infection among adult males.

(d) *Ankylostomum* is more chenopodium resistant than *Necator*.

2. *Chenopodium oil as a vermicide*.—*Chenopodium* oil is a powerful drug which acts as a direct poison to the hookworms. The dead worms retain their natural shape and are never found contorted. Signs of decomposition appear on the second day.

With a dose of 24 minims the ascaricidal action of the oil was noted in 37 cases out of 91 infected. Of 47 *trichuris* infected cases, not one passed worms.

3. *Purging as an aid to chenopodium oil treatment*.—All the 232 cases mentioned below were given 24 minims of oil in one dose, as we had ascertained this dosage to be perfectly safe. Moreover, this being somewhat below the optimum dosage, would lend itself to the study of possible variations due to the purgative.

(a) In 57 cases the drug was preceded and followed by a purge. All cases had from one to four fluid motions on the first day, but seven of them (12 per cent) passed no worms. It was further noticed in this series that rice konjee taken soon after the purgative favoured the moving of the bowels, diminished the giddiness and on the whole made the treatment less exhausting.

(b) 53 cases received no pre-purge. They all had from one to three fluid motions on the first day, but thirteen of them (25 per cent) passed no worms.

(c) 56 cases received no after-purge. Of these 49 had from one to three semifluid motions on the first day, and 32 (57 per cent) did not pass worms. All cases had normal motions next day.

(d) 66 cases were treated without purge either before or after treatment and were given food two hours after the administration of the drug. 29 cases had no motion that day and the rest had one, rarely two, semifluid motions, and 57 (86 per cent) passed no worms. All cases had normal motions next day.



(c) Whatever the mode of treatment, ascarids are never passed on the first day but are mostly expelled on the second day.

4. *Chenopodium oil as a poison to the host.*—Dosages up to 24 minims are safe. The toxic symptoms appearing with this dosage are of a mild character and the giddiness never goes to the extent of preventing the patient from walking about during the treatment. Beyond 24 minims the toxic action increases with the dosage; giddiness is more and more marked, becomes associated with burning of hands and feet, while retching and vomiting occur with the higher dosages.

5. *Efficiency of the chenopodium oil treatment.*—Table VIII records the percentages of cures obtained with one test treatment. Though perfectly aware of the limitations of the method, we still believe that the results based on hookworm removal are sufficiently accurate to allow comparison between the degree of efficiency of the various treatments.

TABLE VIII.

*Efficiency of chenopodium treatment.*

Test treatment.	How given.	Number of cases treated.	Number of cases cured with a "test treatment."	Percentage of cases cured with a "test treatment."
8 minims .. ..	In one portion	17	7	41·2
Do. .. ..	In three portions	19	4	21·0
16 Do. .. ..	In one portion	17	7	41·2
Do. .. ..	In three portions	20	6	30·0
24 Do. .. ..	In one portion	52	19	36·5
Do. .. ..	In three portions	19	9	47·3
32 Do. .. ..	In one portion	20	11	55·0
Do. .. ..	In two portions	11	3	27·2
Do. .. ..	In three portions	18	10	55·5
40 Do. .. ..	In three portions	18	5	27·7
48 Do. .. ..	In three portions	23	9	39·1

6. The 'residue oil' in 48 minims, viz., the amount contained in two and a half drachms of the original oil, removed three per cent of the hookworms harboured by 25 cases and showed no toxic action.

7. *Discussion of results.*—(i). There is a gradual improvement in efficiency with increase of dosage.

(ii) For the same dosage, the one dose treatment is more efficient than the two or three doses treatment. (Tables VI, VII & VIIa.)

(iii) 32 minims in one portion or 48 minims in three portions may be taken as representing the optimum dosage.

With this dosage we have reached the largest worm removal associated with a toxicity below the host's limit of tolerance to the drug.

For simplicity's sake we propose to call "optimum dose" that dosage of oil which shows the maximum toxicity against the parasites together with perfect safety for the host. That the optimum dose may vary for the same oil is shown in Table IX.

TABLE IX.

*Oil IV administered in three portions.*

Age of the oil.		Dose.	Number of cases treated.	Percentage of hookworms removed.	Toxicity.
2 months	..	40 minims..	8	94.9	+ + + +
4	"	20 " ..	6	93.5	+ +
11	"	40 " ..	18	93.1	+ + ±
12	"	48 " ..	23	93.7	+ + +
15	"	24 " ..	19	78.2	+
15	"	8 " ..	19	62.1	0
16	"	32 " ..	18	83.1	+ +
16	"	16 " ..	20	64.2	±

When first used, the oil was as usual ordered in 48 minims doses to be administered in three portions. The toxic symptoms developed after the administration of the second portion were, however, of such a nature as to induce us to reduce the third portion to 8 minims; nevertheless the results were alarming. The total dose of 40 minims was thus far above the host's tolerance. A month and a half later the oil in 20 minims dosages gave satisfactory results, removing 93.5 per cent of the worms without any serious symptoms. We may conclude that at the time (July 1918) the optimum dose was about 20 minims in three portions. (Ascaridol = 70 per cent.)

Wrench<sup>41</sup> (June and July 1918) using the same oil in 48 minims doses divided in three portions records that it is a dangerous drug and is far from safe; in 151 treatments given by him there were 32 cases of vomiting, many cases of giddiness and occasionally slight collapse. On nine occasions two portions only could be given as a treatment, on one occasion one only.

The same oil after a lapse of one year could safely be given in 48 minims dosages. (Ascaridol = 66 per cent.)

The age of the oil has been calculated from the time of its delivery to us, 14th March 1918.

(iv) *Chenopodium* oil is a powerful vermicide.

8 minims administered in one dose remove as much as 74 per cent of the hookworms.

(v) Whatever the mode of administration and the dosage, *Ankylostomum duodenale* is more *chenopodium* resistant than *Necator americanus*.

(vi) Purgation may safely be omitted with sub-optimum doses.

Darling, Barber and Hacker have noted that "the omission of the initial purge did not seem to have any effect in lowering the efficiency of *chenopodium* oil, and may, therefore, be omitted under certain circumstances." The pre-purge has already ceased to be part of the routine treatment adopted by many. From our results as recorded in Table X (b) it would appear that this suppression of the pre-purge does not appreciably affect the efficiency of the treatment.

The purpose of the after-purge is to wash out the unabsorbed portion of the toxic drug and eventually to expel the dead worms which might decompose in the intestine and thus cause toxæmia. By the time we had completed our study of the absorption and elimination of the oil and established that, with the dosages we have adopted, no *chenopodium* was to be found in the feces, we had become sufficiently familiarised with the properties and dosage of the sample to risk the omission of the after-purge without any fear of untoward results. As many as 117 cases received no after-purge and nevertheless revealed no signs of toxæmia. However, judging from the number of worms expelled, it is almost certain that in 61 cases which received no purge at all (Table X) many of the worms must have decomposed and been digested in the intestine.

(vii) *Chenopodium* oil has no vermifugal action.

TABLE X.  
*Anthelmintic action of chenopodium oil with and without purgatives—24 minims in one portion.*

Experiment number.	Test treatment.	Number of cases treated.		HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A TEST TREATMENT.			Percentage of cases cured of hookworm infection with a test treatment.
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.	
a.	With a pre- and after-purge.	52	Test treatment ..	81	1,438	1,519	60.9	83.4	81.8	36.5
			Subsequent treatments ..	53	285	338				
			Total hookworms..	134	1,723	1,857				
b.	With no previous purge	49	Test treatment ..	46	1,150	1,196	35.1	85.0	80.6	37.5
			Subsequent treatments ..	85	203	288				
			Total hookworms..	131	1,353	1,484				
c.	With no after-purge.	56	Test treatment ..	43	1,092	1,135	56.9	88.2	86.6	47.0
			Subsequent treatments ..	30	145	175				
			Total hookworms..	73	1,237	1,310				
d.	With no purge	61	Test treatment ..	29	590	619	?	?	?	57.3
			Subsequent treatments ..	25	140	165				
			Total hookworms..	54	730	784				

44 per cent of the no-purge cases had no motions on the day of the treatment, and the others one motion only. In the absence of the after-purge the dead hookworms were mostly evacuated on the day following treatment. [Table XI, (c) and (d).]

TABLE XI.

*Percentage of hookworms removed on each day of "test treatment" with chenopodium oil—24 minims in one portion.*

Experi- ment number.	Test treatment.	Number of cases treated.		1st day of treatment.	2nd day of treatment.	3rd day of treatment.	4th day of treatment.	Total hookworms removed.
a.	With a previous and after-purge.	54	Number of hook- worms	1216	651	20	0	1887
			Percentage to total	64.4	34.6	1.0	..	..
b.	With no previous purge.	53	Number of hook- worms.	1608	342	14	0	1964
			Percentage to total	81.8	17.5	0.7	..	..
c.	With no after-purge	56	Number of hook- worms.	373	728	34	0	1135
			Percentage to total	32.8	64.1	3.0	..	..
d.	With no purge ..	65	Number of hook- worms.	185	410	63	0	658
			Percentage to total	28.1	62.2	9.5	..	..

(viii) Full diet abates the toxic symptoms without lowering the efficiency of the oil.

Darling, Barber and Hacker have observed that "when the starvation is increased beyond a certain point a remarkable drop in the percentage of worms removed was noted."

Chenopodium acts as a muscular depressant causing a diminution of the functional capacity of the intestinal muscles and this condition must naturally be intensified by purgation and starvation. We have seen that purging could be dispensed with. What then about the diet? As shown by a series of 66 cases who received no purge and were allowed food two hours after the administration of the drug, starvation is responsible for the severity of the toxic symptoms. Full diet cases were very much less giddy than the others and bore treatment well. With the suppression of the after-purge the worm removal ceases to be a sign of efficiency, but if the percentage of cures be taken as an index, we

$\bar{d}$  (obtained from  $d$ 

	0 IMS.	24 MINIMS.	
	ree ions.	One portion.	Three portio
			95·4
			9 case 97·9(1
			4 case
			88·7(
			10 case
			.
6·5			
ases		81·8	78·2
		52 cases	19 case

No	Authors.	PEOPLE EXPERIMENTED ON.			
		Nationality	Sex.	Age.	Health.
1	The Rockefeller Foundation International Health Commission (1915)	Trinidad	?	?	Selected unicariasis patients. Some treated with thymol previously.
	" "	Guatemala	Community work only		
	" "	Trinidad			
	" (1916)	Grenada		"	"
	" "	St. Lucia		"	"
	" "	Costa Rica		"	"
	" "	Guatemala		"	"
	" "	Nicaragua	Males.	Adults.	Healthy army recruits
	" "	Salvador	Community work only.		
	" "	Fiji and Seychelles		"	"
	" Java			"	"
	" Ceylon (1917)	Bogawantalawa		"	"
	" "	Norwood		"	"
	" "	Matale		"	"
	" "	"		"	"
2	Wrench, G. T. (1918)	Indians	Males.	Adults	Sepoys as patients in the hospital
3	Caius and Mhaskar (1917-1919).	Tamil Indians	Males.	Adults 20-50 years.	Healthy prisoners.
	" "	"	"	"	"
	" "	"	"	"	"
	" "	"	"	"	"
	" "	"	"	"	"

TABLE XII.

Percentage of hookworms removed after one treatment with varying dosages of *Chenopodium* oil (obtained from different sources) differently administered.

No.	Authors.	PEOPLE EXPERIMENTED ON.				HOOKWORM INFECTION.		8 MINIMS.		12 MINIMS.	16 MINIMS.		20 MINIMS.	24 MINIMS.		28 MINIMS.	32 MINIMS.			40 MINIMS.	48 MINIMS.	REMARKS.
		Nationality.	Sex.	Age.	Health.	Infection.	Species formula.	One portion.	Three portions.	Three portions.	One portion.	Three portions.	Three portions.	One portion.	Three portions.	Three portions.	One portion.	Two portions.	Three portions.	Three portions.	Three portions.	
1	Schuffner and Vervoort (1913).	?	?	?	?	?	?														84.0	( <sup>1</sup> ) Chloroform 48 minims was given with the third portion of <i>Chenopodium</i> oil.
																					7 cases 91.0( <sup>1</sup> ) 146 cases 60.0 ? cases	
2	Tijssen, J. (1915).	?	?	?	?	?	?															
3	Darling, Barber and Hacker (1918).	Chinese, Malayese.	Males.	Adults.	Healthy prisoners.	58 hookworms per case.	88 Necators to 12 Ankylostomes			80.0 10 cases							95.8 25 cases	96.1 8 cases			98.9 17 cases	( <sup>1</sup> ) <i>Chenopodium</i> oil extracted from proprietary capsules.
	Do.	"	"	"	"	"	"							95.4 9 cases 97.9( <sup>1</sup> ) 4 cases							66.4( <sup>2</sup> ) 10 cases	( <sup>2</sup> ) Given proprietary capsules as such.
	Do.	"	"	"	"	"	"							88.7( <sup>3</sup> ) 10 cases								( <sup>3</sup> ) Half an ounce of liquid petrolatum given with each portion of <i>Chenopodium</i> oil.
4	Knowlton, R. H. (1919).	Carolina and Florida Whites and Negroes.	Males.	Adults.	Convalescent patients.	?	?									78.2( <sup>1</sup> ) 35 cases						( <sup>1</sup> ) Soft proprietary gelatin capsules.
	Do.	Whites only.	"	"	"	?	?									93.4( <sup>2</sup> ) 33 cases						( <sup>2</sup> ) <i>Chenopodium</i> oil extracted from soft proprietary ( <sup>1</sup> ) capsules and placed in hard gelatin capsules.
5	Claus and Mhaskar (1917-1919).	Tamil Indians.	Males.	Adults.	Healthy prisoners.	55 hookworms per case.	94 Necator to 6 Ankylostomes.													98.3 10 cases		Cheno. oil No. 0, 'Good.'
	Do.	"	"	"	"	"	"														88.3 75 cases	" " No. I, 'Old.'
	Do.	"	"	"	"	"	"														96.6 69 cases	" " No. II, 'Fresh.'
	Do.	"	"	"	"	"	"														85.6 25 cases	" " No. III, ' ? '
	Do.	"	"	"	"	"	"						93.5 6 cases								94.9 8 cases	" " No. IV, ' Fresh and good.'
	Do.	"	"	"	"	"	"	74.8 17 cases	62.1 19 cases		77.0 17 cases	61.2 20 cases		81.8 52 cases	78.2 19 cases		96.3 20 cases	87.5 11 cases	83.1 18 cases	93.1 18 cases	93.7 23 cases	" " No. IV, ' One year old.'



must admit that starvation is far from lowering the efficacy of the oil. (Table X(d), column 11.)

(ix) Both the anthelmintic and toxic properties of chenopodium oil are due to ascaridol.

The residue oil and ascaridol glycol administered in doses of 48 minims removed 3 per cent and 1 per cent of the worms, respectively, and had no toxic action. The tarry water insoluble substance, obtained with the glycol by reduction of ascaridol, was also given in 48 minims doses and showed neither anthelmintic nor toxic properties.

### SECTION B.

In the course of this investigation we have used five different samples of oil with notable variations in the results. These variations bear on the removal of hookworms, the number of cures, and the toxic symptoms developed by the patients.

All the five samples exhibited powerful vermicial properties, but for the same dosage under the same conditions of administration the percentage of hookworms removed and the percentage of cures effected were found to vary in proportions wide enough to call for remark.

The most striking variations also occurred in the display of toxic symptoms. Giddiness was general, but manifested in varying degrees of severity. Some cases never went beyond retching, others actually vomited. Not only was diminished hearing noticed, but also temporary deafness, and three cases we eventually lost sight of were still deaf at the time of their release from jail, six and eleven months after the treatment. Of 37 women treated at Dindigul, 12 showed uterine troubles: the drug bringing back menstruation after menopause in three of them, causing metrorrhagia in eight, and abortion in one, two months' pregnant.

The freakish behaviour of the oil is not to be wondered at when we bear in mind that one at least of the constituents is a very potent drug and that no two samples can be expected to hold the same proportion of it.

The want of uniformity in the composition of chenopodium oil is responsible for the conflicting opinions as to its value in the treatment of hookworm infections: opinions which range from wholesale condemnation to boundless commendation. The drug is taken exception to on the score of its toxicity, but praised on that of its efficiency. Its field

TABLE XIII.

Percentage of cures obtained after one or two treatments with varying doses of chenopodium oil differently administered.

No	Authors	PEOPLE EXPERIMENTED ON.				HOOKWORM INFECTION.			CURES OBTAINED AFTER ONE TREATMENT.																CURES OBTAINED AFTER TWO TREATMENTS.						REMARKS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
		Nationality	Sex	Age	Health.	Infection.	Species formula	Method of diagnosing hookworm ova infection.*	8 MINIMS.		10 MINIMS.	16 MINIMS.		20 MINIMS.		21 MINIMS.	24 MINIMS.		30 MINIMS.	32 MINIMS.			36 MINIMS.	40 MINIMS.	45 MINIMS.	48 MINIMS.	16 MINIMS.	20 MINIMS.	30 MINIMS.	36 MINIMS.		42 MINIMS.	48 MINIMS.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
									One portion.	Three portions.	One portion for three days 'Keith method.'	One portion.	Three portions.	One portion.	Two portions.	Three portions.	One portion.	Three portions.	Three portions.	One portion.	Two portions.	Three portions.	Three portions.	Three portions.	Three portions.	Three portions.	Three portions.	Two portions.	Two portions.	Three portions.		Three portions.	Three portions.	Three portions.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
1	The Rockefeller Foundation International Health Commission (1915)	Trinidad			Selected chronic patients. Some treated with thymol previously.	?	?	Examined seven days after treatment.			34.1 133 cases					28.5 130 cases			36.5 74 cases																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													

\* All authors, excepting Wrench, have used Howard's centrifuge method

history is one long attempt at reconciling these two adverse factors in the treatment.

1. *Toxicity*.—In 1917, Zeigler<sup>33</sup> confirmed the work of Salant and his collaborators<sup>24, 25, 26, 29</sup>, who had noted the depressant or irritant effects of the drug on the circulatory, respiratory and renal mechanisms, and on the unstriped musculature of the intestine. And Hall<sup>26</sup>, in 1918, established the gastro-intestinal irritant action of oil of chenopodium already observed by Bruning<sup>12</sup>, in 1906.

Coomarasamy and De Costa<sup>32</sup> credit the drug with a neurotoxic action and report giddiness, ringing in the ears, pains in the joints, as general symptoms after chenopodiol treatment. They further state :— (1) We have come across cases of abortion and miscarriage after a course of treatment with chenopodium oil ; (2) patients suffering from gonorrhœa in a sub-acute form have developed symptoms of arthritis after treatment ; (3) in syphilitic cases, secondary symptoms are ushered in rapidly after the use of this drug ; (4) in certain cases of nephritis, symptoms of uræmic coma have supervened ; (5) in other cases, such as diarrhœa, chronic dyspepsia and malaria, symptoms of the respective diseases have been temporarily aggravated.

Darling, Barber and Hacker have noted dizziness, muscular incoordination, inability to rise, burning in stomach, headache, tingling of the hands and feet, vomiting, and deafness. They also admit that the drug seems to have an action in accentuating a latent gonorrhœa.

Roth<sup>39</sup> says that of 103 patients who were given the oil 29 showed signs of reaction. Dizziness, nausea and vomiting, headache, deafness and general depression were the symptoms observed. Deafness is by far the most disagreeable after-effect of the chenopodium treatment. It occurs in 20 per cent of all the cases, varying in intensity from very mild to a complete loss of hearing, and lasting anywhere from one week to several months. In four of the cases, some deafness still persists two years after the date of treatment.

2. *Dosage*.—The dose most in favour has been 3 c.c. or 48 minims as proposed by Schüffner and Vervoort. It was known to many as the "therapeutic dose" and considered as non-toxic. For reasons not stated in the reports, different dosages have been adopted by the various workers of the Rockefeller Commission. Later on, as the result of a systematic study of the dosage of chenopodium oil undertaken by Darling, Barber and Hacker, the International Health Board<sup>40</sup> (Rockefeller Foundation) concluded that the maximum doses recommended in

the literature were unsafe, and they advised 1·5 c.c. divided in three doses of 0·5 c.c. each, as the standard treatment for hookworm diseases. At present, in Ceylon, the maximum dose figures as 24–28 minims.

48 minims seems to be the maximum dose ever used. Though we think that with such an unsafe drug as chenopodium oil it is better to fail by defect than to err by excess, we still believe that with some samples of oil, larger doses might be administered with perfect safety.

3. *Dispensing*.—Numerous attempts at modifying the mode of dispensing the oil were made with one or more of the following ends in view: enhancing the efficiency of the drug, lowering its toxicity, simplifying the method of administration, making the medicine more pleasant to take and consequently more popular with the patients.

There is no sufficient proof that the addition of oil of eucalyptus, either alone or with chloroform, increases the efficiency of this drug. Chloroform, as will be seen later on when we treat of that substance as an anthelmintic, may have a marked action, but its presence adds to the difficulty of dispensing. Castor oil, at first credited with the power of diminishing the toxicity of chenopodium, not only was found wanting but was shown to intensify the symptoms of giddiness. The anthelmintic effect of chenopodium was greatly impaired by the addition of liquid petrolatum. Soft gelatin proprietary capsules have universally been condemned, but freshly filled hard gelatin capsules are said to be satisfactory.

We need not insist on the fact that a change in the method of administration cannot affect the strength and potency which vary with different samples of the drug.

4. *Treatment*.—Better results have been obtained by varying the mode of treatment. The suppression of the initial purge has rendered the chenopodioid treatment by far less exhausting, and may be regarded as the first step in the right direction. The combination of croton oil and chenopodium oil, which unites both purgative and anthelmintic into a single treatment, is reported to have given satisfactory results.

#### CONCLUSIONS.

1. Oil of chenopodium is a mixture in which the several constituents are present in varying proportions. It deteriorates with age and may easily be adulterated.

2. Oil of chenopodium is highly toxic.

3. Oil of chenopodium is a powerful vermicide acting both on ankylostomes and necators.

4. The toxic and vermicidal properties reside in the same active principle and are, therefore, interdependent.

5. The optimum dose is different for every sample and, so far, there is no accurate and simple method for determining it.

6. Under the conditions there will always subsist an element of doubt as to the safety of the treatment which will necessitate hospital conditions.

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THEIR THERAPEUTIC VALUES IN  
CONNECTION WITH THE  
HOOKWORM INQUIRY  
IN THE MADRAS  
PRESIDENCY.

BY

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III. OLEUM ABSINTHII.

COMPOSITION AND PROPERTIES.

OIL OF WORMWOOD is obtained by the distillation of *Artemisia absinthium*. This plant, indigenous to most European countries, was introduced into the United States of America where its dried leaves and flower-tops were formerly recognised as official under the name of *Absinthium*.<sup>(2)</sup> It was much in vogue as a stomachic tonic, antiperiodic, and anthelmintic, but is at present very seldom used.

*A. absinthium* covers extensive tracts of country in Kashmir and Ladak, and is distributed to North Asia and Afghanistan. Of the numerous other species of *Artemisia* found throughout the mountainous districts of India the following also enjoy some reputation as anthelmintics :—*A. maritima*, *A. persica*, *A. sieversiana*, *A. vulgaris*.

Wormwood oil is usually dark green, sometimes yellow or brownish or even blue, having the strong aromatic odour of the plant, an acrid peculiar taste, and the specific gravity 0.925 to 0.955. The cheaper qualities are occasionally adulterated with alcohol or oil of turpentine. The chemical composition was first systematically investigated in 1845, when Leblanc showed that the principal constituent has the formula

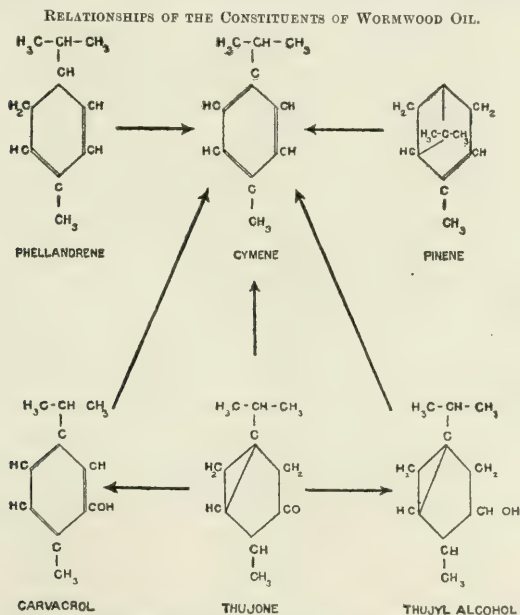


$C_{10}H_{16}O$ . This was confirmed by other investigators, and by Beilstein and Kupfer, who gave to the product the name absinthol and identified its dehydration product with cymene. Later Semmler proved absinthol to be a ketone identical with the essential principles of other oils, as oil of thuja (thujone), tansy (tanacetone), and sage (salviol). (3). (4)

Wormwood oil, as at present established, is composed of thujone; thujyl alcohol, both free and combined with acetic, isovaleric, and palmitic acids; phellandrene, and possibly pinene; cadinene; and a deep blue oil of as yet undetermined composition, but identical with the blue chamomile oil from *Matricaria Chamomilla*. (3), (4)

It is worthy of notice that experiments conducted in the laboratories of Schimmel & Co. (5) have indicated that thujone—and possibly borneol—occurs in the ethereal oil of *Artemisia vulgaris* from India.

Thujone when heated with phosphorus pentasulphide or zinc chloride yields cymene; when heated with ferric chloride it readily forms



carvacrol. Though isomeric with camphor, it differs essentially from that body in chemical reactions.

In large doses wormwood oil is a violent narcotic poison ; in a man, a half ounce caused insensibility, convulsions, foaming at the mouth, and a tendency to vomit. (1)

The sample of oil we used throughout this investigation was purchased from a reliable firm in America. It was a very dark green oil, and afforded the following results : specific gravity (29°C.) = 0.9353; solubility in 80 per cent alcohol = 1:1.5 ; acid number = 7 ; ester number = 102. It contained per cent : hydrocarbons, 15 ; free alcohol, 11.8 ; thujone, 18.2.

#### ABSORPTION AND ELIMINATION.

The colour of the urine after treatment with oil of wormwood varied from pale straw yellow to yellow. Its odour was not in any way characteristic, but on acidifying with hydrochloric acid and heating a very strong thymy smell recalling that of the oil was perceived. The presence of glycuronic compounds did not prevent decomposition.

Wormwood oil is, at least partially, eliminated through the kidneys in conjugation with glycuronic acid, and the elimination takes place during the 24 hours which follow the treatment.

The urines were acid in reaction, and their volume as well as their density were generally normal. No albuminuria was found to have occurred.

#### ANTHELMINTIC VALUE.

Starting with 2 minims, the dose prescribed (6), we treated healthy male convicts with increasing amounts of oil so as to determine the limit of tolerance of the patient to the drug while noting the hookworm removal. The oil was shaken with gum acacia emulsion at the time of administration and dispensed in two portions at an hour's interval. Epsom salts both preceded and followed this test treatment and no food was allowed until the bowels had moved.

The total hookworm content was determined in the usual routine way, using 60 grains thymol as a standard treatment.

The results showed that :—

(a) Toxic symptoms became evident with a 35 minims dosage which produces giddiness, much the same both in nature and intensity as that induced by 24 minims of fresh chenopodium oil.

(b) In a series of 24 cases treated with doses varying from 2 to 35 minims, only five hookworms were removed out of a total of 1,124.

Number of hookworms removed by one test treatment of Wormwood oil.

Experiment number.	Test treatment.	Number of cases treated.		HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A TEST TREATMENT.		
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.
1	2 to 14 minims	14	Test treatment ..	1	3	4	2.9	0.5	0.6
			Subsequent treatments by 60 grs. thymol.	36	587	623			
			Total hookworms..	37	590	627			
2	16 to 20 minims	4	....	..	..	..	..	..	..
3	25 minims ..	4	Test treatment ..	0	1	1	0.0	0.3	0.2
			Subsequent treatments by 60 grs. thymol.	37	347	384			
			Total hookworms..	37	348	385			
4	35 minims ..	2	Test treatment ..	0	0	0	0.0	0.0	0.0
			Subsequent treatments by 60 grs. thymol.	3	109	112			
			Total hookworms..	3	109	112			

(c) No ascarids were removed though round worms were present in five cases and whip worms in four.

It follows from the above that oil of wormwood cannot be recommended as an anthelmintic.

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IV. OLEUM TANACETI.

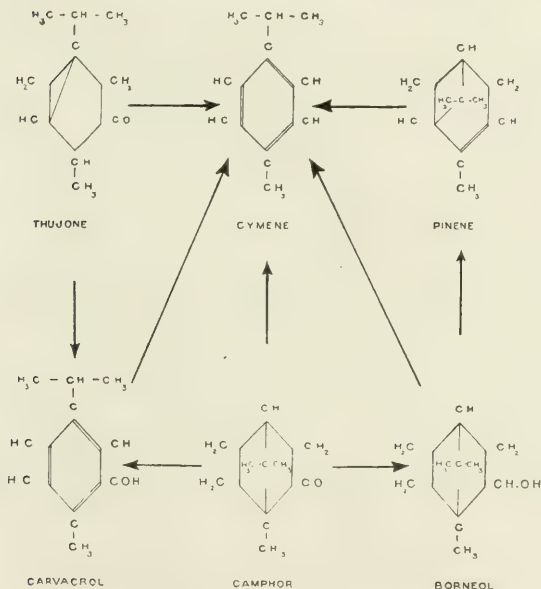
COMPOSITION AND PROPERTIES.

OIL OF TANSY is distilled from *Tanacetum vulgare* whose tops and leaves the United States Pharmacopœia formerly recognised under the name of *Tanacetum*. The seeds of the plant are said to be most effectual as a vermifuge.

About a dozen species of *Tanacetum* are to be found in the Western Himalayas and Tibet, but not one of them seems to be credited with medicinal properties.

Tansy oil is yellow or brownish, with a strong, peculiar, rather unpleasant smell, and a warm, bitter and somewhat acrid taste. Its composition was investigated by Bruylants according to whom it consisted of a terpene, an aldehyde  $C_{10}H_{16}O$ , and an alcohol  $C_{10}H_{18}O$ , (borneol). Semmler ultimately proved that the constituent of formula  $C_{10}H_{16}O$  is not an aldehyde but a ketone, and called it *tanacetone*. It is identical with the ketone found in sage oil (salviol), wormwood oil (absinthol), and thuja oil (thujone). Tansy oil is now generally admitted to be a mixture of thujone, borneol, camphor, and possibly pinene.

RELATIONSHIPS OF THE CONSTITUENTS OF TANSY OIL.



Tansy is said to have, in various cases, produced death. The symptoms caused by it have been abdominal pain, vomiting, violent

epileptic convulsions often followed by profound coma, dilated pupils, great disturbance of respiration, frequent and feeble pulse, and death.

The sample we have been using was a yellow oil with specific gravity (29°C.) = 0.9228; solubility in 80 per cent alcohol = 1 : 2; acid number = 0; ester number = 28. It contained per cent: hydrocarbons, 8; free alcohol, 6.7; thujone, 57.5.

#### ABSORPTION AND ELIMINATION.

Treatment with tansy oil did not affect the density or the volume of the urines, which showed no characteristic colour or odour, and were generally acid in reaction. When acidified with hydrochloric acid and heated they developed a well marked thymy smell, which, however, did not recall that of the original oil. The constituents of tansy oil are therefore, at least partly, absorbed and eliminated through the kidneys in conjugation with glycuronic acid. The elimination does not seem to extend beyond the 24 hours which follow the treatment.

No albuminuria occurred.

#### ANTHELMINTIC VALUE.

Healthy male prisoners were treated with increasing dosages of tansy oil, administered in two portions at an hour's interval. This treatment was preceded and followed by a dose of Epsom salts.

It was first ascertained that 35 minims were required for toxic symptoms to make their appearance. These consisted in vomiting and giddiness, which, in one case, were associated with depression, drowsiness, and a feeble pulse. Later on, while working with 25 minims, a dosage we had every reason to consider perfectly safe, one of the patients developed epileptiform convulsions. He had, however, a good regular pulse and no respiratory distress.

The total hookworm content was determined in the usual routine way using 60 grains thymol as a standard treatment.

The following are the results of our investigation:—

(a) Oil of tansy is toxic in doses of 35 minims; but it would appear that some persons are sensitive to smaller dosages.

(b) The drug is ineffective for the removal of hookworms.

## Number of Hookworms removed by one Test Treatment of Tansy Oil.

Experiment number.	Test treatment.	Number of cases treated.		HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A TEST TREATMENT.		
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.
1	2 to 14 minims	14	Test treatment ..	0	2	2	0.0	0.6	0.6
			Subsequent treatments by 60 grs. thymol.	23	300	323			
			Total hookworms..	23	302	325			
2	6 to 20 minims	4	....	..	..	..	..	..	..
3	25 minims ..	3	Test treatment ..	0	2	2	0.0	2.0	1.8
			Subsequent treatments by 60 grs. thymol.	11	97	108			
			Total hookworms..	11	99	110			
4	35 minims ..	2	Test treatment ..	0	3	3	0.0	4.7	4.6
			Subsequent treatments by 60 grs. thymol.	2	60	62			
			Total hookworms..	2	63	65			

(c) It is equally ineffective for the removal of ascarids, as no worms were expelled though seven cases were found to harbour round worms and two were infected with whip worms.

Tansy oil cannot, therefore, be recommended as an anthelmintic.

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3. ————— .. 1918 The Dispensatory of the United States of America.



## NOTE REGARDING MALARIA IN KASHMIR.

BY

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THE mosquito fauna of Kashmir has not attracted much attention and apart from the information contained in a 'Note of an Enquiry into Malaria and Mosquitoes in the Kashmir Valley.' (1913) by the late Lieut.-Colonel J. R. Adie and Helen A. Adie, there is nothing on record. The reason for this is not far to seek, for in consequence of the rarity of indigenous malaria in Kashmir the usual incentive to the study of its anopheline fauna has been lacking.

But it is inexpedient to neglect either the anophelines or the malarial circumstances of any portion of India and it is therefore proposed to place on record the result of a few observations bearing on these subjects made during a recent short period of sick leave in Kashmir.

It should be said at once that the Kashmir valley did not promise to be a fruitful field of inquiry, for the opinion expressed on all hands was that Kashmir offered an example of a country where there was 'no malaria and no anophelines.' The Adies, it will be seen, arrived at this conclusion which also represents the view of Dr. E. Neve of the Mission Hospital, Srinagar, who speaks from an intimate knowledge of Kashmir extending over a period of some forty years.

### (A)—*The Anophelines of Kashmir.*

The Adies made an exhaustive search extending over a period of six months—from 21st April to 26th October, 1912—throughout an area in the valley about 20 miles long and 10 miles broad which included Srinagar, the capital of Kashmir.

The following species of anophelines were captured :—

- (1) *A. barianensis* (James).—*A. plumbeus* (Haliday) 2 adults caught in the Nasim Bagh, Srinagar, in June, 1912.
- (2) *A. wilmori* (James) 4 larvæ in the Sind valley (August, 1912).

The above authors concluded that these were 'chance captures' and that for all practical purposes the Kashmir valley may be regarded as almost entirely free from anophelines.

In September, 1919, the writer, as the result of observations in the vicinity of Srinagar, found *A. wilmori* breeding in considerable numbers in the numerous small streams which flow into the Dal lake near Srinagar. Over an area of about 8 miles larvæ of all ages (as well as pupæ) were captured in these streams and the irrigation channels in numerous situations.

The species—a proved carrier of malaria in nature—was therefore distinctly abundant in the vicinity of Srinagar in September, 1919.

At Gulmarg (8,500 feet) the writer also captured adults of this species in the houses of Europeans during August and September, 1919. At this time they were biting freely.

Another species—*A. lindesail* (Giles) was also found at Gulmarg. A third species,—*Pt. simlensis* (James)—*A. gigas* (Giles), was breeding freely at Gulmarg in permanent pools. Numerous adults were bred out but none were caught in houses.

These observations show that the anophelines of Kashmir are identical with those found in other parts of the Himalayan tracts. The above observations refer to the Kashmir valley in the vicinity of Srinagar (height 5,200 feet) and to one spur (Gulmarg) raised about 3,300 feet above the general level of the valley.

No observations were made in the valley of the Jhelum river after it leaves Kashmir, from Baramulla (5,100 feet) to Kohala (2,000 feet); at the latter place, however, the following species were captured, the first two being plentiful.

- (1) *A. maculipalpis* (Giles).
- (2) *A. stephensi* (Liston).
- (3) *A. wilmori* (James).
- (4) *A. listoni* (Liston).
- (5) *A. lindesail* (Giles).

*(B)—Malaria in Kashmir.*

The main point of interest attaching to these observations is undoubtedly the fact that *A. wilsoni*—a proved carrier of malaria in nature—was prevalent at Srinagar in the autumn of 1919. It is also worthy of note that it was not uncommon at Gulmarg at a height of 8,500 feet.

In these circumstances it becomes pertinent to inquire what are the conditions at Srinagar in regard to human carriers and to endemic malaria.

In regard to the question of human carriers it may be stated at once that there is no doubt about the presence of human carriers at Srinagar during the summer months, for a large and increasing number of malarious subjects—both British and Indian—visit Kashmir annually at this season either on duty or on pleasure bent.

Relapse cases of malaria are frequently seen both in private and in hospital practice. Adie examined some of these cases at Srinagar and he states that he found evidence of 'a certain amount of imported malaria with sexual parasites in the blood.'

In regard to the question of the occurrence of endemic malaria all authorities are agreed that if indigenous cases of malaria occur at all in the Kashmir valley they must be extremely rare. Walter R. Lawrence, who, as Settlement Commissioner, Kashmir, knew the valley and its inhabitants intimately, states in his classical book 'The Valley of Kashmir' that malarial fevers are unknown in the valley. Dr. E. Neve of the C. M. S. Hospital, Srinagar, speaks perhaps with even greater authority. He states that long experience has proved to him that indigenous cases of malaria do not occur in the vicinity of Srinagar.

Blood films of fever cases admitted to the Mission Hospital are invariably negative in the case of Kashmiris who have never been outside the Kashmir valley. Malaria occurs in the Jhelum valley below Baramulla (5,100 feet) but not in the Kashmir valley. Thus there is a certain amount of malaria at Uri (height 4,000 feet) and at Domel (2,500 feet). He summed up the above views to Colonel Adie as follows:—'It would seem to be largely a matter of altitude, the lower you go the more the malaria.'

Finally, Adie examined between 10th—25th May, 1912, blood films of 101 children (boatmen) living near the Dal lake, Srinagar, but with an entirely negative result. Subsequently, between 22nd August and 15th September, 1912, he examined 79 children of the cultivator class living in the same neighbourhood without finding a single parasite.

Of 18 adults admitted to hospital at Srinagar with fever in only one were parasites found, but this man dwelt at Domel in the Jhelum valley and not in the Kashmir valley.

The available evidence thus points to the conclusion that indigenous malaria is extremely rare if not entirely absent in the vicinity of Srinagar.

In this area, therefore, in spite of the presence of a malaria-carrying anopheline and of human carriers, endemic malaria is conspicuous by its absence.

What explanation can be offered to account for this state of affairs ?

(C)—*Review of the Malaria Factors.*

(1) *The Anopheline Factor.*—It is probable that the prevalence of *A. wilmori* near Srinagar in the autumn of 1919, as compared with the autumn of 1912, was due to the fact that the species is subject to annual variations in prevalence. Indeed, it was asserted that mosquitoes were more common at Gulmarg in 1919 than had been the case for many years. But whatever the normal conditions may be the species was abundant in 1919 and it was not associated with the local incidence of 'fever,' although it is known to be a 'carrier' of malaria in nature.

(2) *The Human Factor.*—It cannot be held that the Kashmiris, of whom about 75 per cent are Mohammedans, possess an immunity to malaria. In the course of a malaria survey of Amritsar in the Punjab in 1913-14 the writer examined the children of a large number of Kashmiri Mohammedans for enlargement of the spleen and malaria parasites with the result shown in the following table:—

*Spleen Rate by Race at Amritsar.*

Race.					Children examined.	Spleen rate.
Punjabis	..	..	..	..	1,353	20
Kashmiris	..	..	..	..	344	18
Hindus	..	..	..	..	1,045	10
Sikhs	..	..	..	..	470	15

The children of Kashmiris living in the Punjab are therefore at least as susceptible to malaria as the children of the indigenous population.

(3) *The Economic Factor.*—It is often asserted that the Kashmiri peasantry live under favourable economic conditions and it therefore might be held that as the result of this circumstance they might offer an unusual resistance to malarial infection. Lawrence states that the chief scourges of Kashmir are famines, floods, fires, earthquakes and cholera. The marked effect of the combination of famine and flooding on malaria in the Punjab is now well known, yet in the history of Kashmir they have been wholly unassociated with widespread sickness. It is noteworthy that, as a result of a partial failure of the rice crop in 1918, there was in 1919 a certain amount of economic stress which showed itself in the poor physique and ill-nourished condition of many of the children. A severe epidemic of cholera also raged throughout the valley during the summer which caused widespread privation amongst all classes.

Economic conditions would therefore appear to have been favourable to malaria in the autumn of 1919.

(4) *Physiographical Features.*—The valley of Kashmir is of an irregularly oval shape, being about 80 miles in length and 20 miles in width. It runs from south-east to north-west.

It is surrounded on all sides by lofty mountains except for a gorge at its north-west extremity through which the Jhelum river, which drains the valley, escapes to find its way into the plains of the Punjab.

The floor of the valley, which consists of a flat, highly fertile plain, is elevated between 5,000—6,000 feet above sea-level, the lowest portion lying between Srinagar and Baramulla.

The soil consists of a recent alluvial deposit of lacustrine origin and yields heavy crops chiefly of rice and maize.

Shallow lakes and swamps cover extensive areas particularly in the low-lying country in the vicinity of Srinagar. The whole valley is well wooded, the Chenar, the Poplar and the Willow being particularly in evidence. Kashmir and particularly Srinagar is famed for its orchards and gardens, the most famous of the latter being that in which

‘The Imperial Selim held a feast

In his magnificent Shalimar.’

(5) *Climatic Circumstances.*—The climate of Kashmir during the summer months is rather hotter than an average English summer. In July and August it is apt to be oppressively hot, shade temperatures of 95° F. being not uncommon.

The mean temperatures at Srinagar during the summer months are as follows :—

		April.	May.	June.	July.	August.	September.	October.
Mean	..	55.0	63.7	74.1	71.4	73.5	70.2	53.3
Maximum	..	65.5	77.7	84.9	86.3	84.6	81.8	63.3
Minimum	..	44.6	51.8	68.2	68.5	62.4	58.6	43.3

The conditions therefore throughout the summer are favourable to the active propagation of malaria through the agency of mosquitoes.

(6) *Meteorology*.—The average annual rainfall is 27 inches, the same as in many parts of the Punjab plains, one-third of which is received in the months of July, August and September.

In regard to humidity the presence of numerous lakes, swamps and streams together with the thick vegetation is responsible for a relatively high degree of humidity, which during the summer months is apt to be oppressive. That the condition as regards humidity are favourable to insect life is shown by the swarms of culicines encountered in the vicinity of Da lake and in the gardens and orchards along its banks.

The average humidity during the summer months at Srinagar is as follows :—

	April.	May.	June.	July.	August.	Sept.	October.
Mean humidity at 8 hours ..	79	74	72	77	77	79	81

#### (D)—Conclusion.

A consideration of all the foregoing circumstances shows that, except in the matter of altitude, no factor or combination of factors can be found to account for the freedom of Kashmir from malaria. Indeed with the above exception, the factors appear to be favourable to the occurrence of endemic malaria in Kashmir.

In regard to the question of altitude, mountainous regions are generally stated to be free from malaria though this statement is rendered obscure by the fact that apparent exceptions to the rule have been reported. Thus Marchiafava and Bignami state that on the eastern slopes of the rocky mountains malaria is found at an elevation of 6,500 feet, whilst, in Italy, Grassi has discovered a malarious district at

a height of 8,400 feet. In India, Quetta (5,500 feet) is stated to be malarious.

Craig concludes that elevation is a protection against malaria only when mosquitoes and the conditions favouring their existence are absent. If such be the case, it would account for the exceptional occurrence of malaria at high altitudes; on the other hand, the conditions in Kashmir being suitable to anophelines, its altitude should be no bar to the prevalence of the disease.

The incidence of malaria at various heights in Kangra and Kulu and in the Simla district in the Punjab is shown in the following table:—

Locality.	Altitude in feet.	No. of children examined.	Spleen rate per cent.	Parasite rate per cent.	Anophelines.
Nurpur ..	2,050	58	65.5	3.5	*1,*2,*3,*4,*6.
Kangra ..	2,419	85	23.0	-	*1,*2,*3,*4,5,*6.
Nagrota ..	2,816	21	33.0	0.0	*1,*2,*3,*4,*6.
Bajjnath ..	3,337	37	16.0	2.7	*1,*2,*3,*4,5.
Magloo ..	3,850	40	2.5	-	*2,*3,7.
Sultanpur ..	4,092	127	22.0	7.2	*1,*2,*3,*4,*6,7
Palampur ..	4,400	166	15.6	3.0	*1,*2,*3,*4,*6,7.
Katraian ..	4,830	25	4.0	0.0	*1,*2,*3,*6.
Banjar ..	5,000	40	2.5	0.0	*2,*3,7.
Gumma ..	5,118	20	20.0	5.0	*1,*2,*3,*4,5,*6.
Naggar ..	5,780	35	5.7	-	*1,*2,*3,*4,9.
Manali ..	6,000	23	nil	nil	*1,*3,*4,*6,7,8.
Chawai ..	6,160	10	nil	nil	*2,*3,7.
Kumar Sen ..	6,560	23	nil	nil	*2,*3,7.
Rawar ..	7,600	13	nil	nil	no record.
Rahla ..	8,000	17	nil	nil	*2,*3,7.
Mathiana ..	8,001	20	nil	nil	nil.
Narkanda ..	8,940	17	nil	nil	*2,*3,7

\*1. *A. fuliginosus*.

\*2. *A. maculipalpis*.

\*3. *A. wilmorei*.

\*4. *A. maculatus*.

5. *A. turkhudi*

\*6. *A. listoni*.

7. *A. lindesaii*.

8. *A. gigas*.

9. *A. plumbeus*.

[\* Malaria carriers in nature.



The absence of malaria in localities having an altitude of 6,000 feet or over, more especially in view of the presence of malaria-carrying anophelines, suggest that the height above sea-level may be the determining factor.

It will also be observed from the table that the critical altitude would appear to be between 5,000--6,000 feet which, it has been mentioned, is the average height of the Kashmir valley.

Unless, therefore, further inquiry should render it necessary to modify the views put forward in this note, it would seem that the cause of the apparent freedom of Kashmir from malaria must be ascribed in some way to its altitude. The exact significance of altitude in its relation to malaria has not been studied and it is therefore proposed in a future communication to endeavour to throw light on this point, which, it is thought, may prove to be possessed of more than academic interest.

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# THE RELATIONSHIP OF MALARIA AND RAINFALL.

BY

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- I.—PRELIMINARY CONSIDERATIONS.
- II.—THE RAINFALL FACTOR IN EPIDEMIC MALARIA.
- III.—THE RAINFALL FACTOR IN NON-EPIDEMIC MALARIA.
- IV.—THE CORRELATION OF FIGURES REPRESENTING 'FEVER' MORTALITY, RAINFALL AND ECONOMIC CONDITIONS.
- V.—THE MECHANISM OF EPIDEMIC MALARIA.
- VI.—CONCLUSIONS.
- APPENDIX I.
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- CHARTS.

- (i) Average monthly rainfall and average monthly 'fever' mortality at Amritsar (1870—1913).
- (ii) Weekly 'fever' death-rate and rainfall figures for the period from July to December in 1881 and 1908.
- (iii) To show the correlation between 'fever' and rainfall at Amritsar.
- (iv) To show the correlation between 'fever,' rainfall and 'prices' at Amritsar.

## I. PRELIMINARY CONSIDERATIONS.

THAT a close relationship exists between malaria and rainfall is a well established fact which has received almost universal recognition

in all malarious countries. From a study of the literature of malaria the following general propositions can be formulated :—

- (1) In the Northern Hemisphere the main malaria season is in the autumn (August to November) whilst in the Southern Hemisphere it lasts from March to May, the autumn in southern latitudes.
- (2) There is usually a slightly increased incidence of malaria in the spring.
- (3) The main malaria season is usually associated with, or follows, the rainy season, where such exists.
- (4) The seasonal oscillations in malaria mortality occasionally exhibit a great amplitude in certain parts of the world, more especially in the subtropical zone. These oscillations are, in the latter areas, usually associated with corresponding oscillations in rainfall; but in other areas, chiefly situated in the tropical zone, a great increase in rainfall may be unassociated with any corresponding increase in the incidence of malaria.

Some of the above points are illustrated in the malarial circumstances of the following localities :—

(1) *Bombay city*,<sup>(1)</sup>—(Climate tropical, spleen-rate 7·2 per cent with local variations between 1 and 30 per cent.) The average annual rainfall of Bombay is 71·5 inches, about three-fourths of which is received during the monsoon period. (June—September.)

The malaria season lasts from July—November and, whilst the malaria mortality is subject to slight annual variations, nothing in the nature of a great epidemic has ever occurred.

In this city an excess of 30 or more inches (40—50 per cent) in the monsoon rainfall produces no appreciable effect on the autumnal fever mortality.

(2) *Muscat*,<sup>(2)</sup> *Arabia*.—(Climate tropical, spleen-rate 58 per cent.)

The average annual rainfall is 4·5 inches. A 'rainy season' does not exist but rain is received in the months of December, January and February. The malaria season, which is ill defined, lasts from November to January. The malaria mortality in April and May is also increased.

(3) *Amritsar City*,<sup>(3)</sup> *Punjab*.—(Climate sub-tropical, spleen-rate variable (15—77 per cent) over a long series of years.)

The average annual rainfall is 23·5 inches, three-fourths of which is received in the monsoon period (July—September).

The malaria season lasts from October to December. There is also a small rise in mortality in April and May. An excess of 10—20 inches (40—80 per cent) in the monsoon rainfall is usually associated with the occurrence of great epidemics of autumnal malaria.

It is thus clear that whilst there is a general association between rainfall and malaria no constant relationship exists between either the total or the monsoon rainfall and the incidence of autumnal malaria; nor does an excess in the monsoon rainfall invariably give rise to a corresponding excess in the autumnal 'fever' mortality.

It is necessary to bear these facts in mind lest, as the result of the study of the effect of rainfall on malaria in any given area, one is tempted to give to the conclusions a general application.

In this communication it is proposed to study the effect of rainfall on malaria in the plains of the Punjab as represented by the city of Amritsar, the malaria circumstances of which have recently been investigated.<sup>(3)</sup>

It has long been recognised in the Punjab that a heavy monsoon rainfall (July—September) is apt to be associated with great epidemics of malaria, which, on account of their sudden onset and severity, Christophers<sup>(4)</sup> (1911) distinguished as 'fulminant.' The latter author, who was the first to study these epidemics by modern methods, clearly established the profound influence exerted by the monsoon rainfall upon their production. Taking the Punjab as a whole during the period from 1868—1908 he found that a high degree of correlation (.67) existed between a series of values representing averages for the yearly monsoon rainfall and a series representing 'fever' deaths in the epidemic season (October and November).

Christophers<sup>(5)</sup> also showed that if the monsoon rainfall for the year be multiplied by a figure representing 'prices' in that year the co-efficient of correlation is as high as .80, whilst an even higher co-efficient (.83) was obtained by multiplying the rainfall and prices figure by a co-efficient obtained by dividing each year's monsoon rainfall by the monsoon rainfall of the preceding year.

Christophers thus demonstrated the important rôle played both by rainfall and economic conditions in the production of fulminant malaria, but he also recognised the existence of another factor or factors for he emphasised the fact that occasionally an excess in the monsoon rainfall had little or no effect on autumnal malaria in the Punjab.

The writer in the report on 'Malaria in Amritsar,'<sup>(3)</sup> 1917, showed that Christophers' conclusions, based on the figures for the Punjab as a whole, were generally applicable to Amritsar, but owing to reversion to military duty he was not in a position at the time to go more fully into the subject.

It is now proposed to rectify this omission.

The summary of conclusions in regard to the effect of rainfall on malaria at Amritsar given in the report was as follows :—

- (1) Excess of rainfall is an important factor concerned in the production of an epidemic of malaria, but excessive precipitation is not the sole determining factor of these epidemics as an abnormally heavy rainfall may be unassociated with severe autumnal malaria.
- (2) The winter and spring rainfall do not appear to exercise any influence on the prevalence of malaria in the following autumn, whilst the monsoon rainfall is of greater importance in this respect than the total rainfall.
- (3) The precise period of rainfall appears to be important, the major epidemics in Amritsar being associated with heavy rainfall continued throughout July and August. The August rainfall is, however, of more importance than that of July.
- (4) The epidemic usually reaches its maximum, as regards mortality in the month of October, but if the monsoon becomes established earlier than usual the maximum mortality may take place in September; on the other hand, if the monsoon is abnormally prolonged the epidemic may reach its maximum in November.
- (5) The precise significance of rainfall and flooding in the mechanism of epidemic malaria is as yet unknown and it can only be determined by observations carried out at the time of epidemic.

In reference to the first conclusion Dr. Gilbert Walker (1919), Director-General of Observatories in India,<sup>(6)</sup> has suggested that the lack of correlation noticeable in certain years between rainfall and malaria at Amritsar may be due to some rainfall factor which has not been recognised and eliminated. And he pointed out that if the July-August rainfall be taken into account, instead of the monsoon rainfall (July—September inclusive), the discrepancies shown to exist in certain

years, notably in the years 1875, 1887, 1900 and 1903, almost entirely disappear.

Dr. Walker showed that the co-efficients of correlation between the rainfall of certain months and the 'fever' mortality in October, and November at Amritsar were as follows :—

June . . .	·21	probable error . . .	·1	approximate.
July . . .	·59	do. . . . .	·07	do.
August . . .	·68	do. . . . .	·07	do.
September . . .	·05	do. . . . .	·1	do.

He thus confirms conclusion (3) quoted above, but from the absence of correlation between the September rainfall and the 'fever' mortality in October and November he concludes that 'other things being equal, heavy rain in September *diminishes* rather than increases "fevers."'

This opinion, if confirmed, is not only of great interest but it demands explanation from the epidemiological standpoint.

As a detailed account of the incidence of malaria at Amritsar during the period 1870–1913 is given in the above-mentioned report, (3) it is not proposed to repeat here the facts therein detailed. In chart I the information necessary to understand the seasonal incidence of rainfall and malaria is depicted.

It will be seen from the chart, which exhibits standard conditions for a large area in the plains of the Punjab, that the main rise in the incidence of 'fever' mortality takes place in September and October. There is a slight decrease in November, whilst during December, there is a rapid decline.

The small 'spring rise' in April and May is also distinct and it is clearly differentiated from the abrupt rise in autumnal 'fever' mortality which commences in September.

The curve of average rainfall precedes, by about one month, the main rise of the 'fever' mortality in the autumn, but there is no corresponding rise in the rainfall curve preceding the spring rise in 'fever' mortality.

## II. THE RAINFALL FACTOR IN EPIDEMIC MALARIA.

From the practical as well as from the scientific point of view, it is of the utmost importance to determine the exact relationship of the monsoon rainfall to the great malaria epidemics, whose periodical occur-

rence at irregular intervals is mainly responsible for the rise in the autumnal 'fever' mortality.

In chart II the weekly fever death-rate per mille for the period from June to December during the years 1881 and 1908—the two great epidemic years at Amritsar—is exhibited together with the rainfall figures by fortnightly periods.

A scrutiny of this chart shows that the epidemics on both occasions commenced with great suddenness during the last week of September and that the maximum was reached in the first week in October in 1881 and in the second week in 1908.

There is slight difference in the mode of decline, for although the epidemic in 1881 was the more severe, the decline was rapid and continuous so that the curve representing the weekly 'fever' death-rate has a 'spiked' appearance, whereas in 1908 the latter resembles a 'saddle-back' type of temperature chart; the decline is also slightly more gradual. The rainfall figures also exhibit some points of interest. Although both epidemics commenced at precisely the same time, in 1881 the June rainfall was 13·2 inches, whilst in 1908 it was 0·3 inches.

The June rainfall would therefore appear to have little or no influence on the *period of commencement* of the epidemic, whilst from the low coefficient of correlation (·21) between the June rainfall and the autumnal 'fever' mortality it is concluded that rainfall in June has little or no appreciable effect on epidemic malaria.

We can thus fix the first week in July as the earliest period in which an excess of rainfall is capable of determining an epidemic.

The latest date in which rainfall could effect the commencement of the epidemics in 1881 and 1908, can also be calculated from the incubation period of the epidemic.

The latter is determined as follows:—

- |   |               |
|---|---------------|
| (1) Sporogony stage in the mosquito     | . 10—12 days. |
| (2) Incubation period of malaria in man | . 10—14 days. |
| (3) Duration of fatal illness           | . 5—30 days.  |

TOTAL	. 25—56 days.
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The average minimum incubation period of these epidemics, as distinct from the minimum possible in individual cases, is therefore 25 days.



It is thus clear that the rainfall responsible for an epidemic which commences in the last seven days of September must have occurred *at the very latest* during the week August 23—29 and that any rainfall which fell after the latter date could not be responsible for the mortality which took place during the first week of the epidemic. Any effect which the September rainfall may exert must therefore be confined to a later period in the epidemic. If its effect on autumnal malaria is proportionate to its magnitude then its influence on the epidemics in 1881 and 1908 was relatively small as the rainfall in September was 4·9 inches and 3·3 inches in 1881 and 1908 respectively. It is therefore concluded that the high ‘fever’ mortality maintained during the later part of the epidemic was *mainly* associated with July—August rainfall.

This view receives support from the detailed report of the 1881 epidemic in which it is stated <sup>(8)</sup> that at the commencement of the epidemic death usually took place within a week whilst in those who died towards its termination the disease had lasted for a month or more.

The rainfall of paramount importance in the causation of malaria epidemics at Amritsar can thus be definitely stated as being the rain received during the period from the first week in July to the fourth week in August.

Thus Dr. Walker’s conclusion that the July—August rainfall is of greater significance than the July—September (inclusive) rainfall in the production of epidemic malaria is fully confirmed from the epidemiological standpoint. It therefore appears permissible to estimate their relative importance by means of comparing their coefficients of correlation which are as follows :—

July—September rainfall .	·73	probable error .	·01	approximate
July—August rainfall .	·81	do.	·01	do.

In these circumstances it is concluded that so far as rainfall is concerned, the July—August rainfall is of paramount importance in the production of malaria epidemics at Amritsar. And it follows therefore—and the point is one of great practical importance—that at the end of August each year the rainfall factor bearing on the production of epidemic malaria in the following October will be definitely known.

### III. THE RAINFALL FACTOR IN NON-EPIDEMIC MALARIA.

From the fact that the rainfall received during the months of July and August is responsible for an epidemic, which reaches its maximum

about the middle of October, it follows that a period of at least 6—7 weeks usually elapses between the rainfall and the *maximum* intensity of the epidemic with which it is correlated. The effect of rainfall on malaria will thus be shown most clearly in the 'fever' mortality figures in the *second* month succeeding the rainfall.

This is indeed in accordance with experience, for in the Punjab the maximum rainfall is usually received in August whilst the maximum 'fever' mortality usually occurs in the month of October. (*Vide* chart I in the case of Amritsar city.)

By means therefore of calculating the correlation coefficients of the rainfall received in any one month, with the 'fever' mortality in the second succeeding month it should be possible to obtain some indication of the effect of rainfall on malaria at all periods of the year. The result of making these calculations is shown in Table I.

TABLE I.

	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
Coefficient of correlation.	+·18	+·15	+·16	+·08	+·11	+·12	+·62	+·69	+·007	+·11	+·02	+·14
Probable error	·09	·09	·09	·10	·09	·10	·06	·05	·10	·09	·10	·10
Standard deviation (rainfall).	1·3	1·4	1·2	0·7	0·8	2·4	4·2	3·8	3·2	0·5	0·1	0·8
Standard deviation ('fever').	119	74	72	100	88	75	76	99	388	838	559	242

NOTE:—The correlation coefficient shown under each month refers to the correlation of the rainfall of the month with the fever mortality of the second succeeding month.

The most conspicuous feature shown in the table is the close association of the July rainfall with the September 'fever' mortality (coefficient of correlation +·62) and of the even closer correlation existing between the August rainfall and the October 'fever' mortality (coefficient of correlation +·69).

The complete absence of correlation between the September rainfall and the November 'fever' mortality (co-efficient of correlation +·007) is also striking and appears to confirm the conclusion of Dr. Walker that an excess of rainfall in September is unfavourable to autumnal malaria.

although, in the absence of a negative correlation, there is no evidence that it actually 'diminishes fever.'

But the low correlation between the September rainfall and the 'fever' deaths in October and November is capable of another explanation. It has been shown that the major epidemics are correlated with the July–August rainfall and that the mortality to which they give rise, which reaches its maximum in October, is continued throughout the month of November. It therefore follows that the 'fever' deaths in November form part of an epidemic, the determining rainfall factor of which is mainly the July–August rainfall.

In these circumstances an absence of correlation between the September rainfall and the 'fever' mortality in both October and November might be anticipated.

The correlation coefficients are as follows:—

October . . . . .	+ '05
November . . . . .	+ '007
October and November . . . . .	+ '05

It is thus concluded that, whilst the September rainfall normally plays little part in the production of epidemic malaria, its exact influence—either in a positive or a negative sense—on autumnal malaria cannot be estimated by means of its correlation coefficient owing to the fact that the mortality associated with the July–August rainfall obscures any effect which might be the result of the September rainfall.

In certain years, however, for reasons which have not been determined, the 'malaria season' in the Punjab occurs somewhat later than usual.

It thus happens that at Amritsar, the autumnal 'fever' mortality which usually reaches its maximum in October, on 14 occasions during the period from 1870–1903, reached its maximum in the month of November.

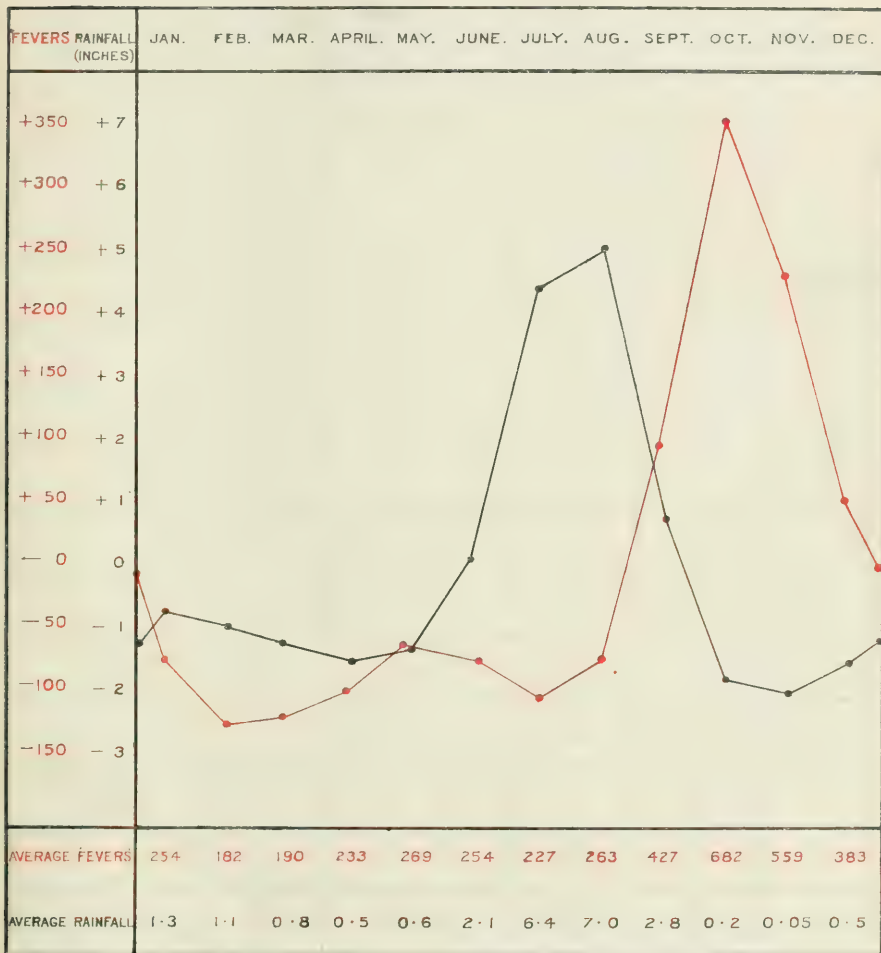
If therefore the September rainfall be correlated with the November 'fever' mortality in these 14 years, it should be possible to determine the influence of the former on autumnal malaria in a manner not possible in normal years.

The following coefficients of correlation between rainfall and the November 'fever' mortality have therefore been calculated:—

August rainfall . . . .	'04	probable error . .	'18	approximate
September rainfall . . .	'43	do. . . . .	'14	do.

# CHART I.

Monthly "Fever" deaths and monthly Rainfall at Amritsar  
1870-1913.



Rainfall

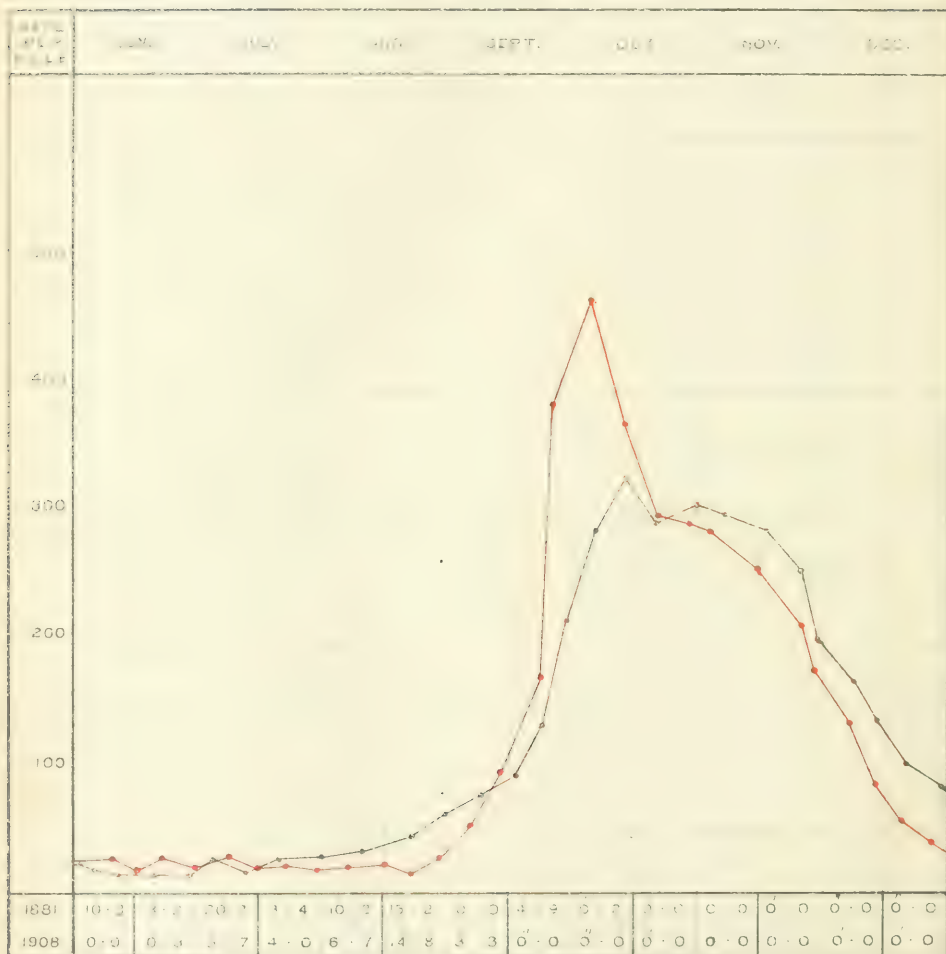
Black.

Fever

Red.

# CHART II.

Weekly "Fever" death-rate in Amritsar from June to December  
in 1901 and 1908.



"Fever" death-rate in 1901

Red.

"Fever" death-rate in 1908

Black.

It is therefore concluded that the September rainfall is capable of influencing in a positive sense the autumnal 'fever' mortality, but from the fact that the average rainfall of this month is small (*vide* chart I) its effect on autumnal malaria is of relatively small account.

It is possible that the 'spiked' nature of the fever curve in 1881 as compared with the 'saddle-back' type of curve in 1908 (chart II) is explained by the fact that in 1881 no rain fell during the first nineteen days of September, whilst in 1908 the monsoon continued until the middle of the month.

Another feature demonstrated in Table I is the fact that there is no obvious correlation between the spring rise in 'fever' mortality (April—June) and the rainfall in the months of February, March and April.

From the above conclusions certain practical deductions may be drawn, but before doing so it is necessary to emphasise the point that malaria has only been considered in so far as it exercises an effect on mortality. Bearing this proviso in mind it can be definitely stated that measures which have as their object the prevention of infection or re-infection with malaria during the autumn require, in the Punjab, to be brought into operation *at the beginning of July*, rather than in August as is more commonly believed. The date of termination of precautionary measures, such as the use of mosquito nets and of prophylactic quinine, cannot be so accurately determined. There is, however, some evidence tending to show that new infections, giving rise to mortality, are not often acquired beyond the end of September or in some years beyond the end of October.

The main season of infection, as distinct from the date of onset of the disease, comprises therefore in the Punjab the months of July, August, September, and October, and of these the two first months are of paramount importance.

#### IV. THE CORRELATION OF FIGURES REPRESENTING FEVER MORTALITY, RAINFALL AND ECONOMIC CONDITIONS.

Utilising the July—August rainfall and the October—November 'fever' mortality and plotting out the figures for each year in the form of a correlation figure we get the result shown in chart III.

This chart demonstrates the marked extent to which the July—August rainfall influences the severity of autumnal malaria at Amritsar; nevertheless in certain years, notably in 1875 and 1912, the rainfall was in excess, whilst the 'fever' mortality was considerably below the mean

in 1912, and scarcely raised above normal in 1875. On the other hand, in the years 1887 and 1902, the 'fever' mortality was out of proportion to the rainfall.

In 1875 the July—August rainfall was 24·9 inches (90 per cent) above normal whilst the autumnal 'fever' mortality was only slightly above the mean. Again in 1887 the July—August rainfall was 16·4 inches (3·3 inches or 25 per cent above the mean) whilst the 'fever' deaths were 2,206 (179 per cent) above the mean. In these circumstances it would appear that the correlation between the July—August rainfall and autumnal malaria at Amritsar is not complete in all years.

The influence of economic conditions can be estimated by means of the method adopted by Christophers in which he multiplies the rainfall figures for the year by the figure representing 'prices' in that year, the latter figure being the number of pounds of wheat purchasable for one rupee. This number is deducted from 60 to convert high prices into high figures. If the figures \* so obtained for Amritsar be correlated with the autumnal fever mortality for the year the result is as follows:

Fever with rainfall  $\times$  prices . . . . . '84 probable error '02.

In chart IV the values are represented graphically. It will be seen that the years 1875 and 1887 no longer stand out so conspicuously as years in which correlation is incomplete; on the other hand in the years 1876, 1881 and 1894 the figures representing 'fever' deaths are no correlated with the rainfall and prices figures.

Again in 1900 and 1912 the figures representing fevers are low as compared with the rainfall and prices figures.

The cost of living in Amritsar has, however, undergone a progressive increase during the past 44 years, whilst it would be incorrect to assume that the 'stress' of living has increased correspondingly. Any influence that may be exerted by economic stress should therefore be more readily appraised, by taking into account *fluctuations* in prices. On these grounds it has been thought expedient to multiply the rainfall and prices figure by a coefficient obtained by dividing the prices of grain in each year by the average price of grain in the two preceding years.

The coefficient of correlation between the figures representing prices rainfall  $\times$  coefficient and autumnal malaria is '57 (probable

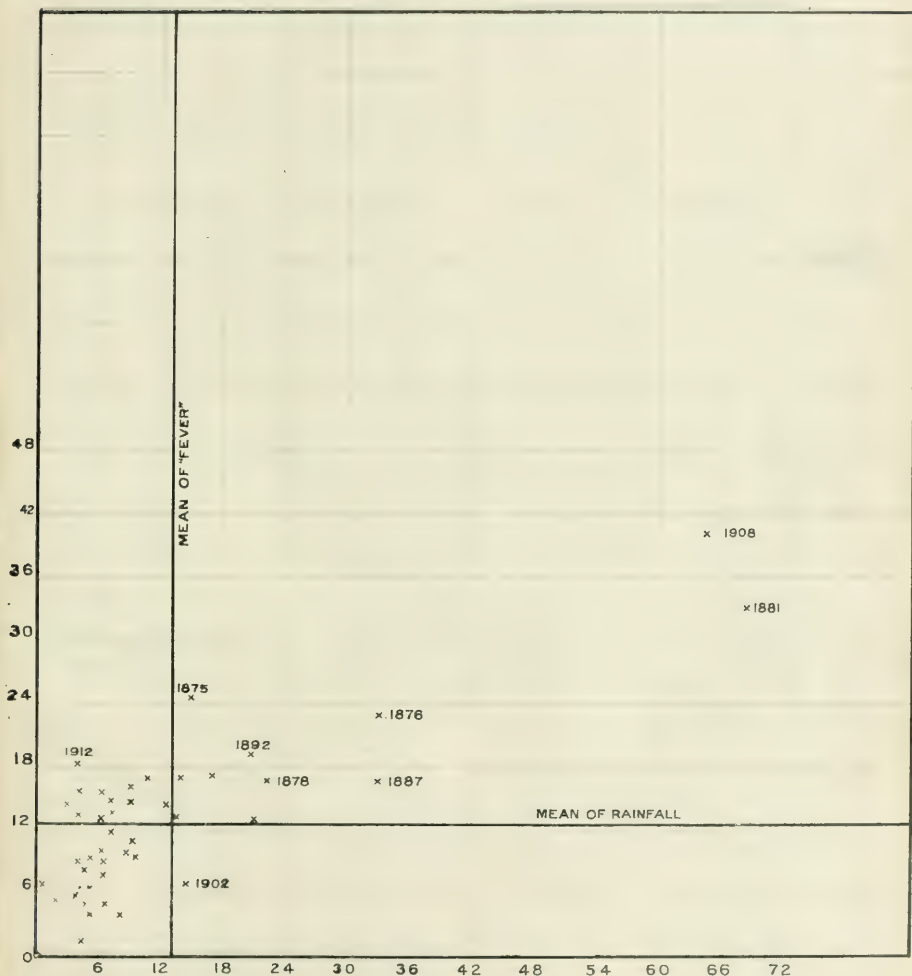
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\* NOTE :—The values, together with the mean and the standard deviation of each series of figures, are given in Appendix I.



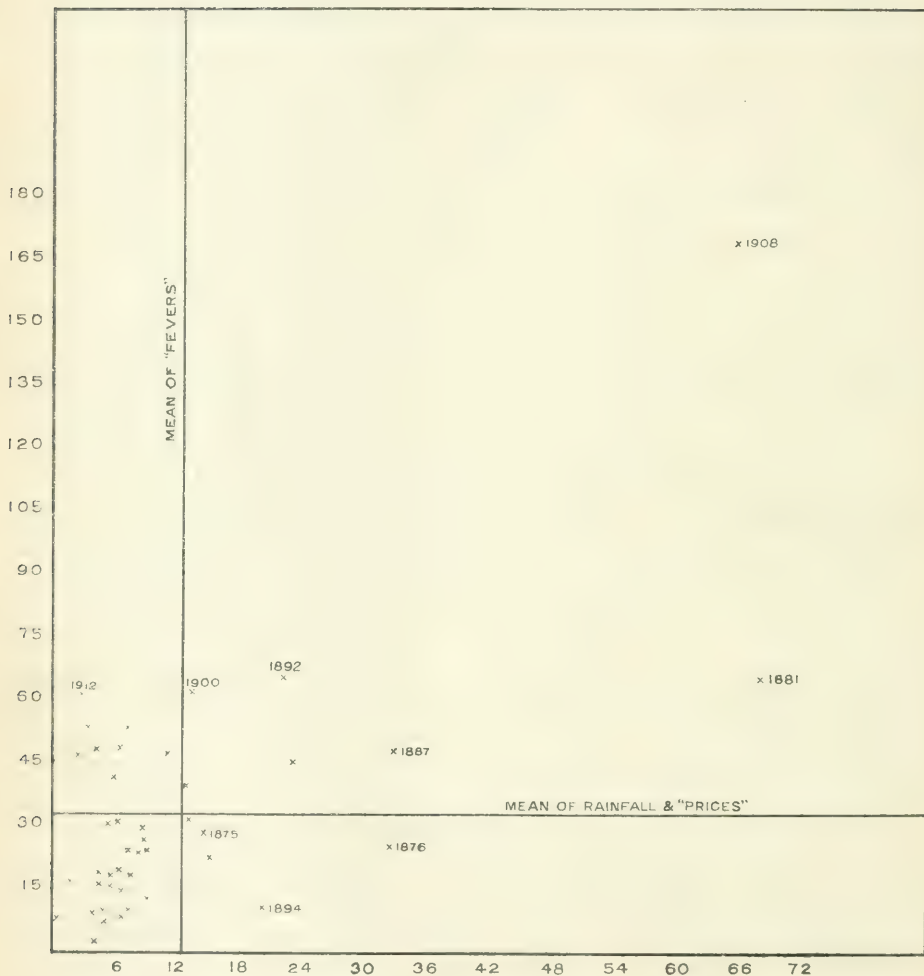
# CHART III

To show correlation between "Fever" and Rainfall at Amritsar.



# CHART IV.

To show correlation between "Fever" and Rainfall and Prices at Amritsar.



error .06), which appears to suggest that the influence of 'prices' has been unduly 'weighted.'

It is therefore concluded that when economic conditions are taken into account the correlation between rainfall and 'fever' is extremely high although in certain years it is still incomplete.

#### V. THE MECHANISM OF EPIDEMIC MALARIA.

The high degree of correlation existing between rainfall and malaria, which becomes even more marked when economic conditions are taken into account, might be considered as implying that malaria epidemics are entirely the result of climatic circumstances associated with droughts and floods giving rise to conditions, in a malarious country, peculiarly favourable to the incidence of malaria. Many circumstances tend to render the view untenable as a complete explanation of the mechanism of these epidemics.

In the first place the correlation between 'fever' and 'rainfall' though marked is not complete and, until the exceptions to the general rule have been explained, it is inexpedient to assume that we are in possession of all the facts underlying their causation.

Secondly, if the excess of rainfall is so profoundly concerned in the production of vast epidemics it would be expected that in all malarious countries a similar result would ensue from a like cause. This, it has been mentioned, is not the case at Bombay, whilst it has been shown elsewhere<sup>(7)</sup> that these great epidemics occur only in certain malarious parts of the sub-tropical zone whilst they are absent from tropical countries even where malaria is often more intense.

Thirdly, although a definite rôle is assigned to economic conditions, its precise significance in the mechanism of epidemic malaria has not yet been explained.

In these circumstances the writer puts forward the hypothesis, in a paper entitled 'Epidemic or Fulminant Malaria together with a preliminary note on the part played by immunity in malaria'<sup>(7)</sup> 1914, that in addition to the rainfall factor and the economic factor, a third factor termed the immunity factor was concerned in the mechanism of these epidemics, and he brought forward evidence tending to show that by postulating the existence of this factor a complete explanation of these epidemics would be forthcoming, whilst their peculiar geographical distribution would also be accounted for.

It is not proposed to enter into this subject here except to state that this study of the effect of rainfall on malaria at Amritsar has brought forward no facts inconsistent with the accuracy of the above theorem.

#### VI. CONCLUSIONS.

1. Whilst in all malarious countries there is a general association between rainfall and malaria, no constant relationship exists between excessive rainfall and the incidence of autumnal malaria.

2. The city of Amritsar, situated in the plains of the Punjab, is an example of a locality where an excess in rainfall is apt to be associated with the occurrence of great epidemics.

3. These epidemics exhibit certain well marked characters; they commence with great regularity and abruptness in the last week in September, reach their maximum in the first half of October and thereafter decline with moderate rapidity.

4. The correlation between the July—September rainfall and autumnal malaria at Amritsar is high, but in the production of epidemic malaria the July—August rainfall is of paramount importance.

5. The influence of the September rainfall on autumnal malaria is of minor importance, but when in excess it is favourable to the incidence of malaria.

6. The rainfall in June, even when the monsoon gives heavy rains in this month, is without apparent effect on autumnal malaria, whilst the effect on malaria of rainfall in the remaining months of the year is also inappreciable.

7. The period when infection and re-infection is most likely to occur at Amritsar commences early in July and lasts until the end of October, the first two months being of paramount importance.

8. In addition to rainfall, economic conditions are capable of influencing the occurrence of epidemic malaria at Amritsar, but, although the rainfall and economic factors are important determining causes of malaria epidemics, the facts are consistent with the view that another factor (or factors) is concerned in their mechanism.

9. As the data regarding rainfall and economic conditions concerned in the production of malaria epidemics in the Punjab are available each year at the end of August it will be possible to forecast with considerable accuracy the occurrences of these epidemics about three weeks before their commencement. Such a forecast would, however, be wholly inaccurate in the case, for example, of Bombay, where

similar rainfall and economic conditions have never given rise to great epidemics.

## APPENDIX I.

	Fever deaths (October and Novem- ber) in hundreds.	Rainfall July— August.	Prices.	Rainfall × Prices.	Coeffi- cient.	Rainfall × Prices × Coeffi- cient.
1870 . . .	4.3	6.0	32	19.2	1.0	19.2
1871 . . .	1.3	6.4	14	8.9	1.0	8.9
1872 . . .	7.9	16.5	16	25.4	0.7	17.7
1873 . . .	9.8	11.7	12	14.0	0.8	11.2
1874 . . .	6.4	8.4	12	10.0	0.9	9.0
1875 . . .	15.2	24.9	12	29.8	1.0	29.8
1876 . . .	34.3	23.0	12	27.6	1.0	27.6
1877 . . .	4.7	2.9	10	2.9	0.8	2.3
1878 . . .	23.2	17.7	26	46.0	2.3	105.8
1879 . . .	9.6	8.8	32	28.0	1.8	50.4
1880 . . .	5.5	6.3	28	17.6	0.9	15.8
1881 . . .	68.1	33.5	20	67.0	0.6	40.2
1882 . . .	7.1	13.7	12	16.4	0.5	8.2
1883 . . .	4.0	8.5	14	11.9	0.9	10.7
1884 . . .	7.0	12.2	8	9.7	0.6	5.8
1885 . . .	4.9	7.9	8	6.3	0.7	4.4
1886 . . .	8.7	14.9	20	29.8	2.5	64.5
1887 . . .	34.3	16.4	30	49.2	2.1	103.3
1888 . . .	11.6	17.1	28	47.8	1.1	52.5
1889 . . .	8.6	14.3	20	28.6	0.7	20.0
1890 . . .	13.0	13.6	24	32.6	1.0	32.6
1891 . . .	5.7	9.7	22	31.0	1.4	43.4
1892 . . .	22.0	19.7	34	66.9	1.2	80.2
1893 . . .	9.1	10.2	28	28.5	0.8	22.8
1894 . . .	20.3	12.8	10	12.8	0.3	3.8
1895 . . .	6.1	7.8	24	18.7	1.2	22.4
1896 . . .	4.5	13.9	36	50.0	2.1	105.2
1897 . . .	6.3	12.6	40	50.4	1.3	65.5
1898 . . .	6.0	14.4	28	40.3	0.7	28.2
1899 . . .	5.2	6.2	28	17.3	0.8	13.8
1900 . . .	13.6	17.7	36	63.7	1.3	82.8
1901 . . .	12.5	13.3	30	39.9	0.9	35.9
1902 . . .	15.5	7.8	28	21.8	0.8	17.4
1903 . . .	17.8	17.9	26	46.5	0.9	41.8
1904 . . .	5.1	4.1	24	9.8	0.9	8.8
1905 . . .	5.2	5.7	30	17.1	1.2	20.5
1906 . . .	6.8	5.0	30	15.0	1.1	16.5
1907 . . .	6.0	9.5	32	30.4	1.0	30.4
1908 . . .	65.5	40.3	42	169.2	1.3	219.9
1909 . . .	7.0	13.2	40	52.8	1.0	52.8
1910 . . .	3.3	14.4	34	48.9	0.8	39.1
1911 . . .	2.4	5.2	32	16.6	0.8	13.2
1912 . . .	4.3	18.5	34	62.9	1.0	62.9
1913 . . .	4.2	15.5	36	55.8	1.0	55.8
Mean . . .	12.3	13.1	25	34.0	1.05	39.1
Standard deviation.	13.9	7.25	9.5	27.2	.45	39.0

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# THE PATHOGENESIS OF DEFICIENCY DISEASE.

## NO. X. THE EFFECTS OF SOME FOOD DEFICIENCIES AND EXCESSES ON THE THYROID GLAND.

BY

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I PROPOSE in this report to summarize the observations I have hitherto made with respect to the influence of imperfect and ill-balanced foods on the thyroid apparatus. In it are included certain experiments dealing with the effects of overfeeding.

The dietaries employed in these researches group themselves, with respect to their influence on the thyroid gland, into two categories: (1) those inducing a diminution in its size and weight, and (2) those inducing an increase in its size and weight.

*Inanition* leads to marked atrophy of the thyroid gland in pigeons. The average weight of both thyroids per kilo of original body-weight was 59 mgs., as compared with 85 mgs. in healthy controls. Histologically the vesicles may be shrunk into various shapes and partially emptied of colloid, their size reduced and the intervacular tissue relatively increased in amount.

### A.—DIETARIES INDUCING A DIMINUTION IN SIZE OF THE THYROID.

1. *An exclusive diet of autoclaved milled rice.*—This diet is deficient in all classes of vitamins, in suitable protein, in fats and in salts, while it is excessively rich in starch. It is possible also that the high temperature of the autoclave destroys other food elements requisite for perfect nutrition. There is in such a dietary a shortage of roughage.



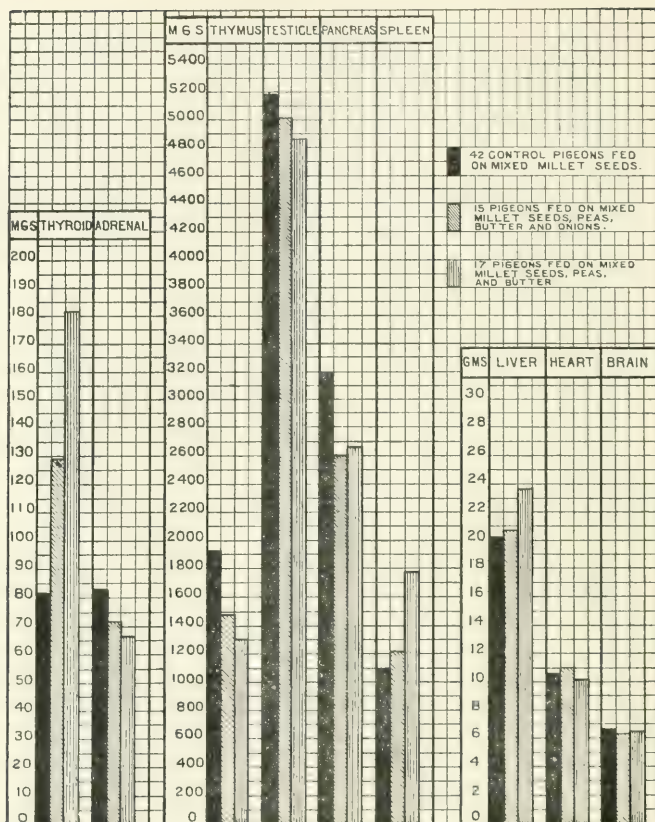


Fig. 1.—Chart showing average weight of organs per kilo of body-weight in control pigeons and in over-fed pigeons. Note the effect of onions in control ling the enlargement of the thyroid, spleen and liver. The smaller size of the adrenals and possibly also the smaller size of the pancreas in butter-fed pigeons is related to the excess of fats in the food. The variations in size of the thymus and testicles in over fed birds is too small to admit of any conclusions. In view of the causation of "goitre-heart" it is to be noted that no enlargement of the heart is associated with the thyroid hyperplasia in these experiments. The pituitary (not included in the Chart) tends to be reduced in weight in over-fed birds.

Its effects on the thyroid gland were studied in thirty-three pigeons and in twelve monkeys (*macacus sinicus*). It gives rise in both species to a moderate degree of atrophy. In one experiment in pigeons the average weight of both thyroids per kilo of original body-weight was 75.9 mgs. as compared with 85.1 mgs. in healthy controls. In monkeys the diminution in weight of the organ is not so marked, owing no doubt to the short time these animals survive an exclusive dietary of autoclaved rice. The thyroid glands in both species presented the same varying

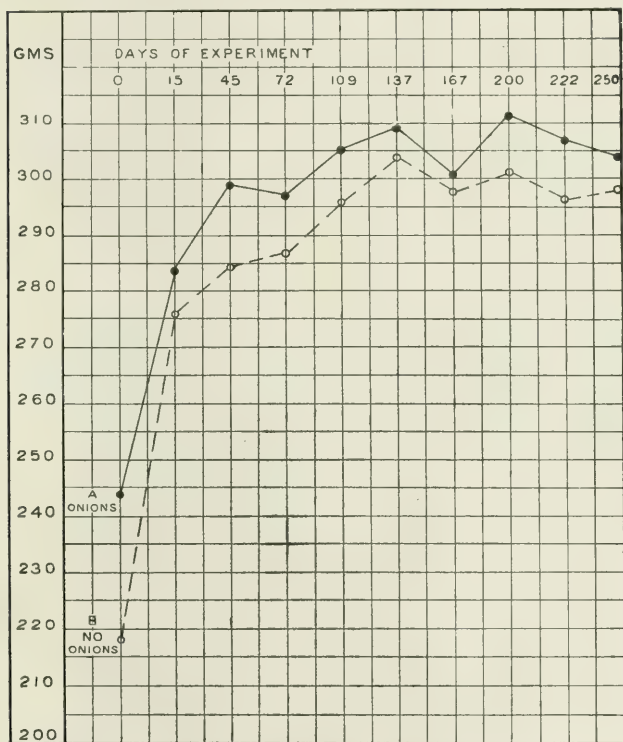


Fig. 2.—Chart showing monthly average weights of two series of pigeons—

(A) Fed on mixed grains with butter and onions.

(B) On the same diet, but without onions.

histological pictures seen in health (Figs. 3 & 4), colloid glands (1) preponderating. The evidences of pathological change were comparatively slight and limited to congestion (Fig. 9) and to a greater proportion of cells showing evidence of necrobiosis. Congestion was not present in all cases; the number so affected in monkeys was five out of twelve. The most notable pathological appearance was found in animals presenting hæmic infections, a not infrequent consequence of this dietary. Then the organ showed marked congestion, desquamation of acinar epithelium, varying degrees of necrosis of parenchyma cells, and complete or partial disappearance of colloid.

2. *A diet of autoclaved rice and butter.*—This diet is deficient in vitamins of the 'B' and 'C' classes and in roughage; it is also excessively rich in starch and in fat. Its effects were studied in four monkeys. The degree of atrophy was considerable having regard to the rapidity with which the animals died (fifteen days) (2). The average weight of the organ was 73 mgs. per kilo of original body-weight, as compared with 83 mgs. in healthy controls. It may here be noted that the amount of thyroid tissue as estimated by weight is the same in healthy pigeons and in healthy monkeys (*macacus sinicus*). In all four cases in this category the glands were of the colloid type (Figs. 3 & 4), or of a type showing a tendency to reversion from the colloid state (Fig. 10) (1). The vesicle walls consisted of a single layer of cuboidal epithelial cells; the acini contained a pale pink staining colloid in which a few vacuoles, indicating absorption of colloid, were present. In all cases, the periacinar capillaries were much distended (Fig. 9), each acinus being sharply outlined, in part of its circumference, by a capillary envelope of pink-staining blood corpuscles. The intervesicular parenchyma was very scanty, and necrobiosis of parenchyma cells was slight. The parathyroids in three cases, in which they were found in sections, were intensely congested (Fig. 10), and in one hæmorrhagic infiltration had caused disruption of the compact masses of polygonal cells composing the gland, and death of many of them. Similar changes in these organs have been encountered on one other occasion only in my experimental experience: in the parathyroids of new born rats whose mothers were fed daily throughout pregnancy on anaerobic cultures of faecal bacteria (3). The cause of the hæmorrhagic infiltration of the parathyroids was probably the same in both instances. In the one, the micro-organisms, or their products, operated through the medium of the maternal blood, in the other, the deficient dietary caused such changes in the intestinal mucosa

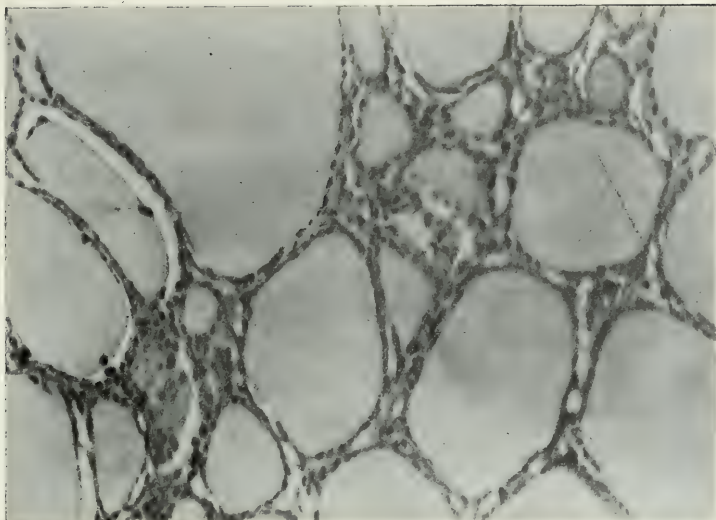


Fig. 3.—Colloid gland, not enlarged, from monkey fed on autoclaved rice. A similar histological picture was seen in 35% of controls. . 265.

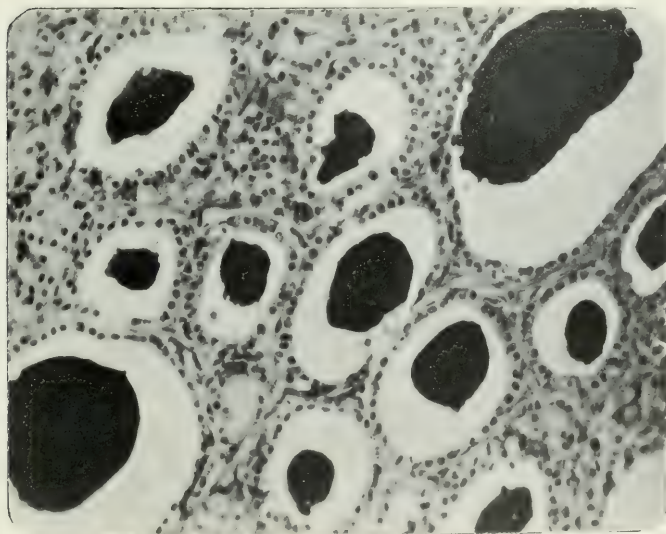


Fig. 4.—Thyroid gland, not enlarged, from monkey fed on autoclaved food, butter and onions. Note proportion of intervesicular tissue to acini.  $\times 265$ . A similar histological picture was seen in several control animals.





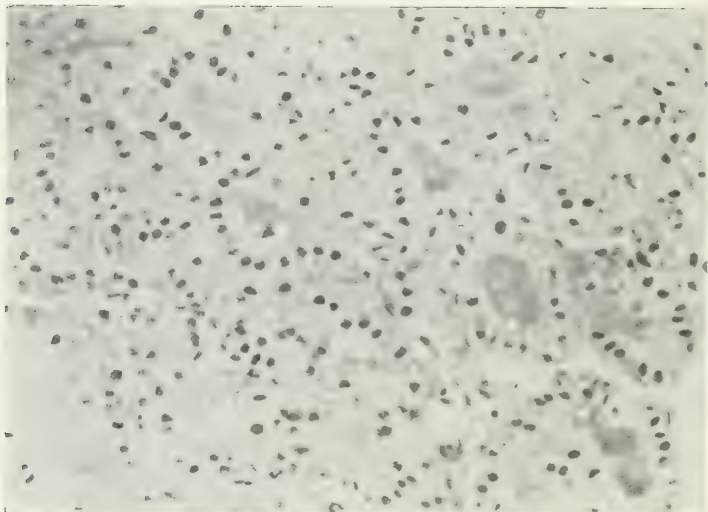


Fig. 5.—Normal actively secreting thyroid from a control monkey. Note increase in height of acinar cells, vacuolation and absorption of colloid.  $\times 265$ .

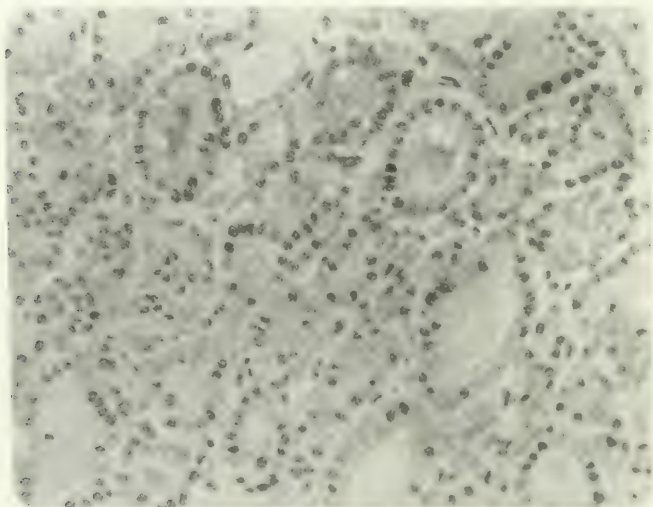


Fig. 6.—Non-enlarged thyroid from a monkey fed on autoclaved food and onion. Note increase in height of acinar cells, increased number of nuclei (Compare fig. 5), vacuolation of colloid and small size of many vesicles.  $\times 265$ .



as subjected the parathyroids to the noxious effects of micro-organisms or of their products, derived from the intestinal tract. It is remarkable that only in this category were the parathyroids found to be notably altered by morbid change. A point of considerable interest in this connexion is that the adrenal medulla of pigeons fed on this dietary was almost invariably intensely engorged and the adrenalin content subnormal.

3. *A diet of autoclaved rice, butter and onions.*—This diet is deficient in 'B' vitamines and excessively rich in fats and in starch. Its effects were studied in twenty-four pigeons. The atrophy of the thyroids was considerable, their average weight amounting to 69 mgs. per kilo of original body-weight, as compared with 88 mgs. in butter-fed controls. Histologically the glands presented appearances similar to those seen in the case of birds fed exclusively on autoclaved rice; congestive and infective changes were, however, less frequently present. It may here be noted that the addition of onions to the dietary did not prevent the onset of avian polyneuritis.<sup>(4)</sup>

4. *A diet of autoclaved food and onion.*—This diet is deficient in vitamins of the 'A' and 'B' classes. Its effects were studied in six monkeys. The atrophy of the thyroid was considerable, its average weight per kilo of original body-weight amounted to 69 mgs., as compared with 83 mgs. in healthy controls. Histologically the thyroid presented more definite evidences of departure from normal than in other categories. Whereas in the control animals the number of thyroids of the colloid type (Figs. 3 & 4) <sup>(1)</sup> exceeded those of the actively secreting type (Fig. 5) <sup>(1)</sup>, in monkeys fed on autoclaved food and onion the histological appearances were in all six animals of the latter type.

In addition to this preponderance of glands of the actively secreting type there was present area for area a greater number of nuclei. Thus the nuclear count in the normal actively secreting gland (Fig. 5) was 175 to a given field, whereas it was 275, 195, 250 and 238, respectively, in four animals fed on autoclaved food and onion. This piling up of nuclei might be regarded as indicative of hyperplasia, but in no case did I detect evidences of nuclear division. A section of the thyroid from one such case is shown in Fig. 6. While, therefore, there is in animals of this category an appreciable loss of weight of the thyroid gland, there is no histological evidence of atrophy of its parenchyma cells; the tendency is rather to hyperplasia of these cells. No notable changes were observed in the parathyroids.

5. *A diet of autoclaved food, butter and onion.*—This diet is deficient in 'B' vitamine. Its effects were studied in five monkeys. The diminution in weight of the organ was considerable, the average being 70 mgs. per kilo of original body-weight, as compared with 83 mgs. in healthy controls. The histological pictures did not differ appreciably from the normal (Figs. 3 & 4). There was a tendency for glands of the actively secreting type (Fig. 5) to predominate. No notable changes were observed in the parathyroids.

There is one factor which is common to these five dietaries—deficiency in vitamines—as there is one result which is common to their use—diminution in size and weight of the gland. It seems reasonable to believe that deficiency of these substances is the cause of the reduction in size of the organ. A further effect of these deficient dietaries is that they expose the thyroid cells to the noxious action of bacteria, and their products, derived from the intestine.

#### B.—DIETARIES INDUCING ENLARGEMENT OF THE THYROID.

1. *A scorbutic diet of crushed oats and autoclaved milk.*—This diet is deficient in 'C' vitamine. It is also deficient in essential salts and roughage. Its effects were studied in five guinea-pigs. The average weight of the thyroid per kilo of body-weight in five controls was 95 mgs. The average weight of the organ in five guinea-pigs fed on the scorbutic diet was 218 mgs. per kilo of original body-weight, and 295 mgs. per kilo of final body-weight, or from two to three times the weight of the healthy organ. This increase in weight was found on histological examination to be due in the main to hæmorrhagic infiltration of the organ. It would be of considerable interest to observe whether the thyroid gland undergoes enlargement in infantile scurvy. I have myself no data in this regard.

2. *A diet of mixed varieties of millet seeds without sand or grit.*—The effects of this dietary were studied in forty-two pigeons. No great departure from normal was observed either by gravimetric or histological methods of examination. The birds appeared to derive from the mixture of millet seeds all the vitamines (including antiscorbutic substances and salts) requisite for the maintenance of normal nutrition. It is true that a few cases (three in number) presented evidences of moderate degrees of hyperplasia—the glands weighing in these cases 45, 42 and 34 mgs. respectively, as compared with an average weight of 24 mgs. But such examples of thyroid hyperplasia are inseparable from

confinement of animals within narrow bounds. These forty-two pigeons, a number of which were under experiment for 250 days, served as controls to the two remaining observations.

3. *A diet of mixed varieties of millet seeds, peas, butter and onions without sand or grit.* The effects of this dietary were studied in pigeons:—

(a) Eighteen young birds, age not exceeding six months, were confined in a single, large, netted-wire bottomed cage ( $\frac{1}{2}$ -inch mesh) of the following dimensions: 5 ft.  $\times$  3 ft.  $\times$  2 ft. Their daily ration consisted of white millet seeds (*cholam*) 12 oz., small dry millet seeds (*ragi*) 8 oz., small green peas of the variety known in India as *mung dal* 8 oz., fresh butter 3 oz., chopped onions  $6\frac{1}{2}$  oz. No sand or grit was provided. The onion was a small local variety, grown in the Madras Presidency, of very pungent odour. The birds were allowed to eat as much of this mixture as they wished. Fresh water was supplied daily in large shallow troughs suspended above the bottom of the cage. The water became very dirty from the birds' droppings and from their habit of bathing in the troughs. The experiment commenced on the 14th April, 1919, and terminated 248–250 days later on the 18th–20th December, 1919. During its course three birds died in consequence of maltreatment by their fellows. The pigeons were weighed weekly, their live weight was taken just before they were killed for examination. Fig. 2 shows the composite monthly average weights of these birds. It will be noted that after a sharp rise up to the fifteenth day, the average weight showed a steady increase up to the end of the experiment.

(b) Eighteen young pigeons, age not exceeding six months, were confined in a cage precisely similar in every respect to the preceding. They received the same food as the birds in the previous experiment but *without onions*. In all other respects the two experiments were identical, both commencing on the 14th April, 1919, and terminating on the 18th–20th December, 1919. The sole difference was the absence of onions from the dietary in the present instance. During the course of the experiment one bird was killed by its fellows. The pigeons were weighed weekly; their live weight was taken just before they were killed. Fig. 2 shows their composite monthly average weights. It will be noted that the weight curve runs parallel with that of birds receiving onions.

The dietary employed in these two experiments was excessively rich in protein and in fats. In the case of the birds receiving onions all classes of vitamins were present in abundance. In the case of the birds receiving no onions, 'C' vitamin was more scanty, but appears

to have been present in quantity sufficient for their needs since the forty-two controls on mixed milled seeds exhibited no evidence of scurvy. There was in the dietary of both categories, as well as in that of the forty-two controls, a deficiency of grit and of mineral substances which the birds pick up in the natural state. In those receiving onions, deficiency of salt and cellulose was largely made good by the onions. The two dietaries presented a further difference: that which did not include onions was made up mainly of acid and neutral foods (the seeds and butter); that which included onions contained a much higher proportion of alkaline foods (onions). It may here be mentioned that endemic goitre is unknown in this locality (Coonoor, the Nilgiris, Madras Presidency, 6,000 feet).

The pigeons in these two experiments were killed on the 248<sup>th</sup>, 249<sup>th</sup> and 250<sup>th</sup> days of the experiment, and their organs removed and weighed. The following table, and the chart (Fig.1) which illustrates it, show the average weight of the organs per kilo of final body-weights as compared with forty-two control pigeons fed on mixed millet seeds.

TABLE.

*Showing average weights of organs per kilo of body-weight in control pigeons and in over-fed pigeons. Duration of experiment—250 days.*

			42 control pigeons fed on mixed millet seeds.	15 pigeons fed on mixed millet seeds, <i>mung dal</i> , butter and onions.	17 pigeons fed on mixed millet seeds, butter and <i>mung dal</i> .
Thymus	..	..	1.862 gms.	1.485 gms.	1.306 gms.
Thyroids	..	..	82 mgs.	128 mgs.	183 mgs.
Liver	..	..	20.286 gms.	20.720 gms.	23.610 gms.
Spleen	..	..	1.081 gms.	1.203 gms.	1.775 gms.
Adrenals	..	..	83 mgs.	72 mgs.	66 mgs.
Testicles	..	..	5.186 gms.	5.068 gms.	4.875 gms.
Pancreas	..	..	3.180 gms.	2.613 gms.	2.671 gms.
Heart	..	..	10.675 gms.	11.026 gms.	10.135 gms.
Brain	..	..	6.62 gms.	6.32 gms.	6.52 gms.



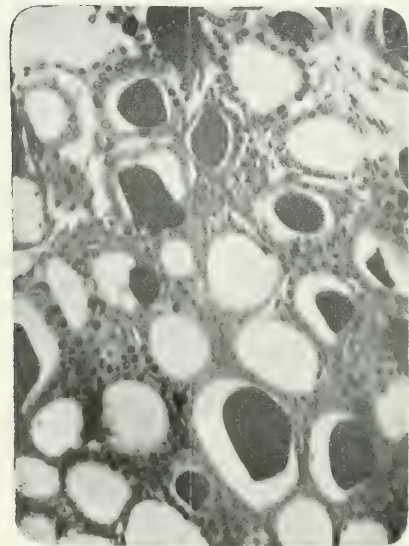


Fig. 7.—Thyroid from healthy control pigeon.  $\times 210$ . Note regular shape and size of vesicles, low columnar acinar epithelium and masses of intervesicular tissue to acini. The masses of colloid have dropped out of most of the vesicles in the process of staining.

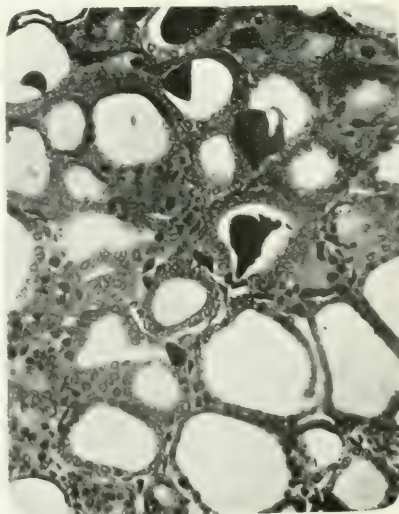


Fig. 8.—Thyroid from control pigeon kept in confinement for 250 days.  $\times 210$ . Note slight departure from normal, increased proportion of intervesicular parenchyma.

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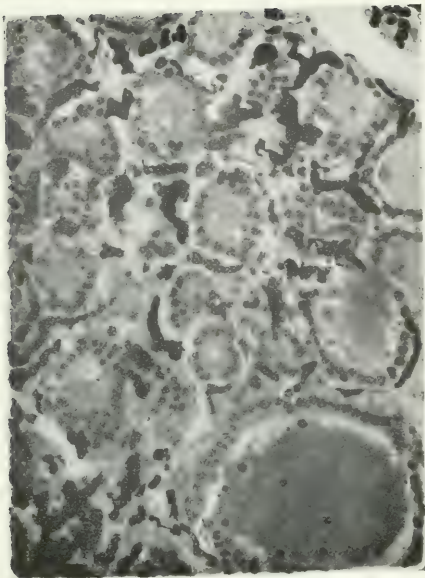


Fig. 9.—Thyroid from monkey fed on autoclaved rice and butter. Note engorgement of periacinar capillaries. The darker staining cells are blood corpuscles.  $\times 210$ .

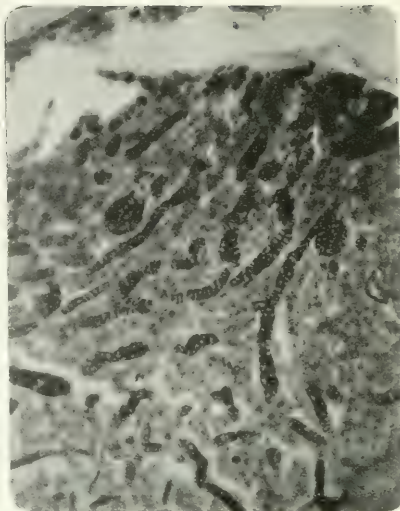


Fig. 10.—Parathyroid from monkey fed on autoclaved rice and butter. Note great engorgement of capillaries.  $\times 210$ .



In control pigeons the weight of the thyroids varied between 15 and 42 mgs. with an average weight of 24 mgs., or 82 mgs. per kilo of body-weight.

In pigeons fed on mixed millet, *mung dal* and butter *with onions*, the weight of the thyroid ranged between 15 and 87 mgs. with an average weight of 39 mgs., or 128 mgs. per kilo of body-weight.

In pigeons fed on mixed millet, *mung dal* and butter but *without onions*, the weight of the thyroid ranged between 16 and 130 mgs. with an average weight of 54 mgs., or 183 mgs. per kilo of body-weight.

Naked eye evidences of thyroid enlargement were rarely met with in pigeons fed on mixed millet seeds, and when present were never excessive. Some degree of thyroid hyperplasia is undoubtedly attributable to the factor of confinement alone. It is difficult, however, to determine the precise value of this goitrogenous factor apart from food influences. The average weight of 82 mgs. per kilo of body-weight in controls is inclusive of any increase in weight of this organ which may be attributable to confinement, and to the absence of gritty mineral particles from the dietary. It follows, therefore, that in pigeons overfed on mixed grains and butter whether with or without onions, any enlargement of the thyroid over 82 mgs. per kilo of body-weight is attributable to the excessive richness of the food in proteins and fats. In those fed on mixed grains and butter with onions the thyroid enlarged considerably, whereas the enlargement was much greater when onions were withheld from the dietary. In the former category the weight of the organ exceeded the highest limit found in controls in five out of fifteen cases, in the latter this limit was exceeded in eleven out of seventeen cases. Thus the incidence of goitre as well as the average weight of the thyroid was greater in animals receiving no onions. In both categories the larger glands presented to the naked eye appearances of hyperplastic organs: they were increased in size, dark in colour, firm in consistency, and appeared to hold little or no colloid. The right was almost invariably the larger of the two, thus conforming to the rule in the human subject. The thyroids were examined histologically in eight cases fed on mixed grains and butter *with onions*, and in ten cases fed on mixed grains and butter *without onions*. Amongst the eighteen thyroids there were five types of histological picture:—

*Type A.*—Glands of normal structure or differing from normal only in a slight or moderate increase of the intervesicular parenchyma, vesicles regular in outline, lined



by regular cuboidal cells and containing eosinophile colloid (Figs. 7 & 8). Glands of this type were met with in two cases amongst the ten from pigeons fed on mixed grains and butter but without onions; they were not enlarged.

*Type B.*—Enlarged glands showing slight or moderate congestion with the formation of many new acini. Acini small in size and irregular in shape, lined by a single layer of cuboidal cells of no greater height than that seen in health, and showing no, or but a slight, tendency to the formation of intra-acinar buds or plications. Colloid scanty or absent and either basophile staining or unstained. Intervesicular tissue was very scanty, having all been utilized for the formation of new vesicles. The multiplication of vesicles is the essential feature of this type (Figs. 11, 12, 13). Glands of this type were met with in five out of eight cases from pigeons fed on mixed grains, butter and onions: no thyroids of this type were found in pigeons receiving no onions.

*Type C.*—Glands, not always enlarged, similar to those of type B, but showing pronounced desquamation of acinar cells. Colloid absent or very scanty and basophile staining. Glands of this type (Fig. 14) were encountered in three cases out of eight fed on mixed grains, butter and onions, and in one case (Fig. 18) which received no onions. The histological picture in this type resembles closely that seen in animals suffering from hæmic infections, or in those which had received experimental inoculations of the toxic products of bacteria.

*Type D.*—Enlarged glands showing slight or moderate degrees of congestion, the formation of many new acini and an almost complete absence of stainable colloid. The acini are very small, and lined by a single or multiple layer of cells which are usually *high* cuboidal in type, or low columnar. There is a marked piling up of nuclei in the acinar wall—the evidence of commencing budding. The small acinar cavity is often

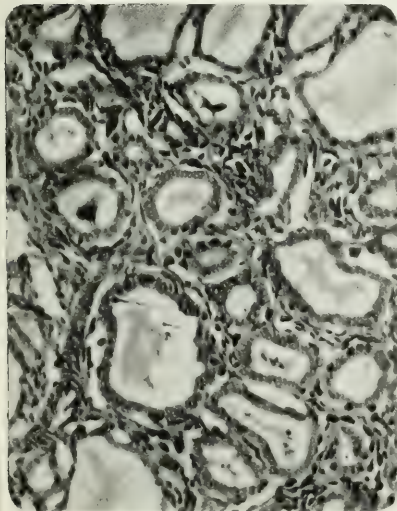


Fig. 11.—Thyroid from pigeon over-fed on mixed grains, butter and onions. Note commencing irregularity in shape of vesicles, and their increased number per field. Colloid basophile. No acinar budding.  $\times 210$ .

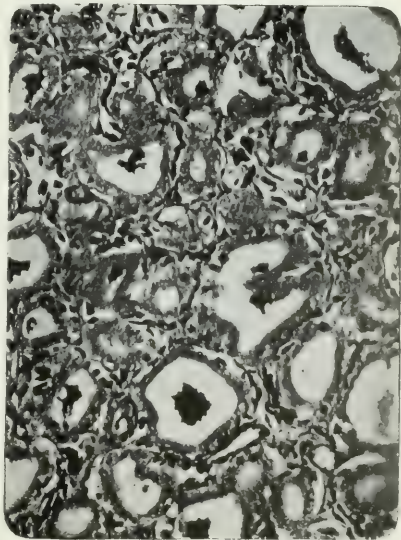


Fig. 12.—Thyroid from pigeon over-fed on mixed grains, butter and onions. Note similar appearances to those seen in Fig. 11, greater number of newly formed vesicles, also intra-acinar 'bud.'  $\times 210$ .

R. MCCARRISON.—The Pathogenesis of Deficiency Disease. No. X.

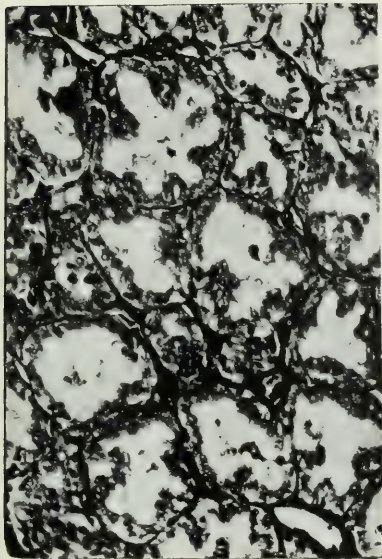


Fig. 13.—Thyroid from pigeon over-fed on mixed grains, butter and onions. Note disappearance of colloid, ragged and disquamating acinar cells and tendency to acinar budding.  $\times 210$ .

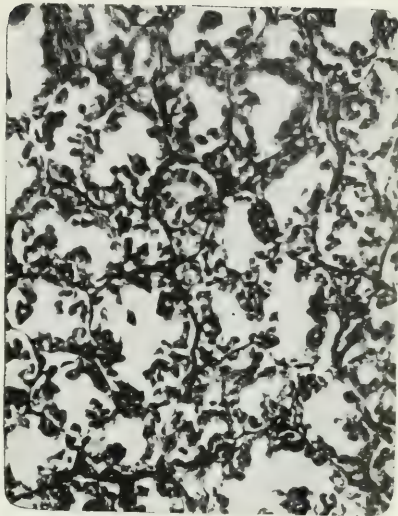


Fig. 14.—Thyroid from pigeon over-fed on mixed grains, butter and onions. Note disappearance of colloid and intense desquamation of acinar cells.  $\times 210$ .

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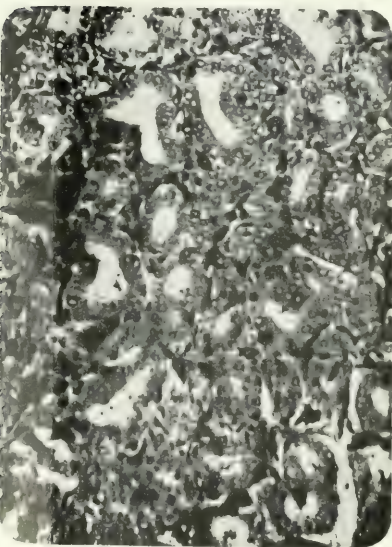


Fig. 15.—Thyroid from pigeon over-fed on mixed grains and butter but *without oats*. Note very small size of acini, disappearance of colloid, increase in height of acinar cells and in thickness of acinar walls, irregular shape of acini and commencing protrusion of acinar walls into acini.  $\times 210$ .

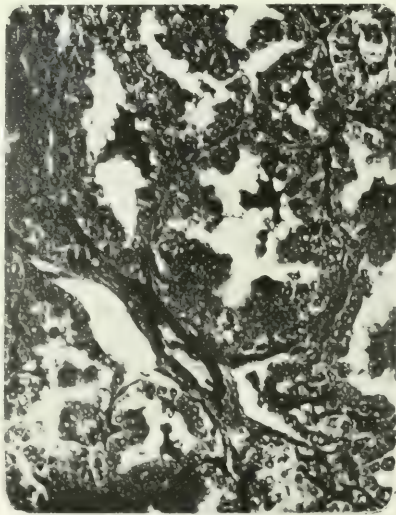


Fig. 16.—Thyroid from pigeon over-fed on mixed grains and butter but *without oats*. Note practical disappearance of colloid, great irregularity in shape of vesicles and marked budding.  $\times 210$ .

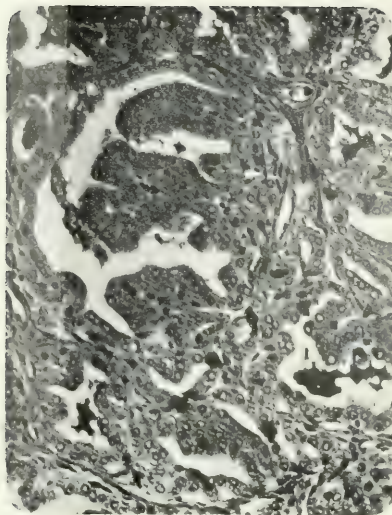


Fig. 17.—Thyroid from pigeon over-fed on mixed grains and butter but *without oats*. Note similar appearances to those seen in Figs. 15 and 17, but more intense building. Appearances identical with those of Graves Disease.  $\times 210$ .



Fig. 18.—Thyroid from pigeon over-fed on mixed grains and butter but *without oats*. Note similar appearances to those seen in Fig. 14, but desquamation associated with budding.  $\times 210$ .



distorted in shape and slit-like (Fig. 15). The organ presents an almost solid appearance in section, colloid being very scanty, and such as is present either retaining the basic stain or failing to stain. Glands presenting this histological picture throughout sections were met with in only one case amongst pigeons receiving no onions. This type represents an earlier stage of Type E.

*Type E.*—Enlarged glands showing the typical histological picture of the thyroid in Graves' Disease. Vesicles small, irregular in shape, and lined by high cuboidal or low columnar epithelium, the vesicle walls being often several cells thick and showing a very pronounced tendency to the formation of intra-acinar buds or plications (Figs. 16 & 17). Stainable colloid absent, or but scanty and unstained or faintly basophile. Glands of this type were found in six cases out of ten fed on mixed grains and butter but without onions.

It is thus seen that the great majority of enlarged thyroids from pigeons, fed on mixed grains, butter and onions, are included in Type B—organs exhibiting abnormal multiplication of acini with cuboidal acinar cells and a *slight* tendency to intra-acinar budding—while the great majority of enlarged thyroids from pigeons fed on mixed grains and butter but without onions are included in Type E—organs exhibiting abnormal multiplication of acini with high cuboidal or columnar acinar cells and with a very *marked* tendency to intra-acinar budding. It is to be noted that the tendency to budding was present in both categories, but that this tendency was much less pronounced when onions formed part of the dietary. The conclusion is thus forced upon one that the onions restrain the tendency to hyperplasia of the Graves' Disease type. It seems probable that *succus alii* might be used with advantage for this purpose in Graves' Disease. In none of the numerous experiments which I have carried out in connexion with the experimental production of goitre have I found this tendency to the production of a Graves Disease type of goitre so predominant as in the case of pigeons fed on a diet excessively rich in proteins and fats and deficient in roughage and vegetable salts.

It will be noted also that in this experiment the chemical reactions of the colloid are altered. The secretion lost its eosinophile staining

character and remained either unstained or retained the basic element of the stains employed.

Here then is a result in marked contrast to that found in the case of dietaries deficient in vitamins. Foods deficient in vitamins cause the thyroid to diminish in size whereas those containing abundance of vitamins, but excessively rich in proteins and fats, cause in pigeons, confined within narrowed limits, an enlargement of the thyroid amounting, in over 50% of cases, to actual goitre. In these circumstances the goitrogenous changes in the thyroid are attributable to confinement, lack of exercise, overfeeding with food excessively rich in proteins and fats, the absence of mineral particles from the food, and it may be of appropriate vegetable salts or other unknown substances which in the natural state pigeons may require for the maintenance of perfect health. It is extremely difficult to narrow the goitrogenous factors in these cases down to a single influence. For my own part I believe that the goitres were due to the combination of a number of unhygienic and nutritional causes. Thus an excessive proteid diet alone, all other hygienic conditions being perfect, will not, as Forsyth<sup>6</sup> has shown, cause thyroid hypertrophy. In the present experiments, overfeeding was the main causal agent in the production of the goitres, since pigeons fed only on millet seeds, similarly confined and deprived of mineral gritty matter, did not exhibit thyroid hyperplasia to any great degree. This is not to be taken to indicate that overfeeding *per se* is a cause of goitre, but rather that imperfect balance of the food and its over-richness in certain directions *when associated with unhygienic conditions of life* is a potent factor in the production of goitre.

The factor of duration of exposure to the goitrogenous influence is of considerable importance in determining the size of the thyroid enlargement. In an experiment where twenty-four pigeons were fed for forty-three days on a dietary of mixed grains, butter and onions, the average weight of the thyroid per kilo of body-weight was 88 mgs., whereas in pigeons fed on the same dietary for 250 days it reached 128 mgs. The degree of thyroid hyperplasia is thus in some measure proportionate to the duration of exposure to the goitrogenous influence which, in this instance, was overfeeding.<sup>5</sup>

We come now to consider the most remarkable and, I think, the most important result of this experiment, *viz.* the effect of the onions in restraining the tendency to the thyroid hyperplasia, as well as the tendency to the pronounced acinar budding



characteristic of goitre in Graves' Disease. This effect may have been due—

- (1) to enrichment of the dietary in iodine which onions may have afforded ;
- (2) to the action of the antiseptic juice contained in the onions ; and
- (3) to the increased richness of the food in vegetable salts and cellulose when onions formed part of the dietary. .

With regard to the first of these possibilities I have no chemical evidence to offer either for or against it. But the experiments themselves provide evidence which appears to indicate that the thyroid hyperplasia was not the result of deficiency of iodine in the food. For, when pigeons were fed on *autoclaved rice*, butter and onions, no thyroid hyperplasia occurred. Whereas when pigeons were fed on *mixed grains*, butter and onions, hyperplasia resulted. Any deficiency in iodine would, it may be presumed, be more marked in the former case. Further, when monkeys were fed on autoclaved food, butter and onions, no enlargement of the thyroid resulted. It may be assumed that the autoclaved food contained less available iodine than the unautoclaved grains fed to pigeons in the present experiment. Although the factor of time is not the same in these cases, it may, I think, be concluded that the goitre was not the result of lack of iodine in the food : the determining factor in its production would appear to have been the overfeeding with mixed grains and fat. With regard to the second possibility, the action of oil of garlic as a stimulant, stomachic and antiseptic, and its value in tubercle, pneumonia and chronic bronchitis, are well recognized. Amongst native hakims in India onions are credited with a curative and prophylactic action in cholera. It is reasonable then to suppose that the onions used exerted a considerable influence on the character of the bacterial flora of the intestinal tract. In the present experiment this influence may have been due in part to the pungent juice in the onions ; it was probably also connected with the maintenance of a more normal H-ion concentration of the bowel contents, by virtue of the alkaline salts they contributed to the food. The grains themselves being rich in protein and belonging to the class of foods yielding an acid ash are likely to promote the preponderance in the bowel of a bacterial flora of putrefactive (anærobic) types, and especially so when confinement and unhygienic conditions of life favour the entry of such bacteria into the intestinal tract ; whereas the addition of onions to the dietary of mixed

grains and butter provides the requisite alkaline elements of a properly constituted food. Thus the harmful effects of the high protein-content of the grains may have been corrected in considerable measure, and the tendency to the preponderance of noxious types of putrefactive flora correspondingly restrained. It seems probable also that the more generous provision of salts in the case of onion-fed pigeons served to maintain a more normal metabolism and a more normal state of health and of permeability of the intestinal mucosa. Whatever be the mode of action of onions, whether by virtue of their salt or antiseptic content or both, one of their effects is to render the conditions in the gastro-intestinal tract less favourable to the absorption from it of bacterial toxins, or of poisonous products of proteid cleavage. For, amongst the most important of the functions of a well-balanced food, are (1) the maintenance of healthy functional activity of all elements of the gastro-intestinal tract, and (2) the maintenance of a normal intestinal flora. The effects of food deficiencies and excesses on the body tissues cannot be adjudged apart from these fundamental facts.

The chart (Fig. 1) showing the comparative weights of the organs in the two classes provides additional evidence of the noxious effects of the products of intestinal bacteria on the body tissues and of the influence of onions in restraining them. It will be noted that in birds receiving no onions, not only was the thyroid greatly enlarged but the liver and the spleen also, a circumstance which may, I think, be attributed to the greater toxicity of substances absorbed from the intestinal tract. This experience provides further evidence of the very important part played by intestinal influences in the genesis of goitre and of its congenital manifestations and of the inter-dependence of nutritional and bacterial factors in their production—a truth which I have amply demonstrated by previous epidemiological, therapeutic and experimental methods of study.<sup>1\*</sup>

#### CONCLUSIONS.

1. Dietaries deficient in vitamins lead to a reduction in size and weight of the thyroid gland.

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\* The attention of the reader is especially directed to my papers "On the experimental production of Congenital Goitre" *Proc. Roy. Soc., London*, 1916, B, LXXXIX, pp. 322-327, 1 pl. and *Ind. Journ. Med. Research*, 1916, IV, No. 1, pp. 183-189, also "Experimental Researches on the Etiology of Endemic Cretinism, Congenital Goitre and Congenital Parathyroid Disease," *Ind. Jour. Med. Research*, 1914, I, No. 3, pp. 505-522.—R. McC.

2. Dietaries deficient in vitamins render the thyroid gland susceptible to the noxious action of intestinal bacteria, or of their products, with resultant atrophic and necrotic changes.

3. A scorbutic diet of crushed oats and autoclaved milk may cause in guinea-pigs considerable enlargement of the thyroid gland. The enlargement is, in the main, the result of congestion and hæmorrhagic infiltration of its tissues.

4. Dietaries containing adequate provision of vitamins, but excessively rich in proteins and fats, induce in the thyroid gland of pigeons in confinement marked degrees of hyperplasia, the extent of the hyperplasia being largely dependent on the duration of the organs' exposure to the goitrogenous influences induced by the excessive protein and fat content of the food.

5. The addition of onions to a dietary excessively rich in protein and fats, while containing at the same time an abundance of vitamins, markedly retards the development of thyroid hyperplasia, and the tendency to acinar 'budding' in pigeons living in confinement. The beneficial influence of the onions is held to be due in part at least to their action in restraining the growth of putrefactive types of bacteria in the gastro-intestinal tract and in retarding the absorption of their products. It is suggested that *succus alii* might prove of benefit in restraining the thyroid hyperplasia of Graves' Disease.

6. The changes in the parathyroids induced by a diet deficient in vitamins and excessively rich in starch and fat appear to be related in their origin to intestinal anaerobes, the noxious action of which is greatly favoured by the defective diet.

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# SECRETION AND EPITHELIAL REGENERATION IN THE MID- INTESTINE OF *TABANUS*.

BY

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THE cellular changes which are associated with the processes of digestion and absorption of food in the mid-intestine of insects have been described by several writers, dealing with insects of different orders. The mechanism of secretion appears to proceed on much the same lines in all, whether the food is solid or exclusively fluid, and subsequent workers have in the main confirmed the original observations of van Gehuchten in the larva of *Ptychoptera*. The blood-sucking Diptera, however, both on account of the special nature of their food and because of the habit, almost universal among them, of feeding only at relatively long intervals and then taking an amount of food which is very large in proportion to their size, present certain special features which are of considerable interest. Although the cellular changes which occur during the functional activity of the epithelium of the mesenteron have been fully described as they occur in the Ticks, the corresponding processes in the blood-sucking Diptera have received little or no mention in recent works dealing with the minute anatomy of the group. It is the purpose of the present paper to describe the processes of secretion and epithelial regeneration as seen in *Tabanus*, a fairly typical member of the order.

The majority of the flies used for experiment were *Tabanus albimeditus*, Walker—a large species. Both caught and bred specimens were used. The caught specimens were taken just as they had finished a meal under natural conditions—the host being invariably a cow—and

were kept in large cages in the laboratory for the desired period subsequent to the meal. The bred specimens, for which the writer was indebted to Major Patton, were fed by inverting the large test-tubes in which they were confined on the skin of the host.

*The Alimentary Tract.* (Plate LXI, figure 1.)

The alimentary tract of *Tabanus* is of a simple type. The first portion, situated within the head, is modified to form the sucking apparatus, which consists of two chitinous chambers; strong muscles pass between the walls of the chambers and the head capsule, and provide the motive power for the sucking pump. A short *œsophagus*, which is of narrow calibre and has a well developed chitinous intima, runs through the neck and connects the sucking pump with the mid-intestine. The *mid-intestine* lies in the thorax and anterior part of the abdomen, and consists of two easily distinguishable parts. The first of these is the *cardia*, a tubular structure, flattened dorso-ventrally in its first part, and expanded at its commencement into two lateral masses; the surface is mammillated, the small elevations being most conspicuous in the first part of the organ. The second part, which, since it corresponds to the part in the mosquito known by that name, may be called the *stomach*, is of much greater diameter, and is in fact the chamber into which the blood taken in during a meal is received; when empty its surface has a roughened and irregular appearance, due, as will be seen presently, to the heaping up of the epithelium into villi. The mid-intestine terminates abruptly at the point at which the Malpighian tubes open into the alimentary tract. The *hind-gut* may be divided into an *ileum*, which forms a simple loop in the posterior part of the abdomen, a *colon*, of slightly greater diameter, running straight backwards to open into the pear-shaped *rectum*, which has six rectal papillæ of the usual type.

The *œsophageal diverticulum*, or *crop*, is a small bi-lobed sac with delicate walls. It lies in the abdomen, and is connected with the *œsophagus* by a long and narrow duct. This duct joins the *œsophagus* exactly at the point at which the latter enters the upper end of the *cardia*: in fact, when the structures are traced in serial sections of the whole fly the duct of the crop appears as the direct continuation of the *œsophagus*.

*Mode of Feeding.*

*Tabanus* is generally quiet and deliberate in its mode of feeding. Having selected a suitable spot on the skin of the host it inserts its mouth

parts and usually takes a full meal before they are withdrawn. The meal may last as long as three or four minutes. As the stomach becomes more and more distended, the abdomen increases in size, until the dorsal and ventral plates become separated sufficiently to disclose the connecting membrane. The distension, however, is not so great as is the case in the mosquito. During the meal the abdomen exhibits slow rhythmical contractions, which are probably due to peristaltic movements of the wall of the alimentary tract. Towards the end of the act a few drops of a dark fluid—the remains of the last meal which have accumulated in the rectum—are passed from the anus. Later a few droplets of a clear fluid exude, and in some instances a little red and apparently unaltered blood.

If a fly is dissected immediately after it has fed, the whole of the meal is found in the stomach. The cardia is empty, and appears by comparison as little thicker than the stalk of a pear. The crop of the recently fed fly is never found to be distended with blood, though its walls, which are well supplied with muscle fibres, almost always show slow but strong rhythmical contractions.

Under natural conditions *Tabanus* appears to feed every two or three days. It can be kept alive for a considerable time in captivity if fed on alternate days. Great difficulty was experienced in inducing many of the bred specimens to feed. It was found that bright sunshine is essential, and that it is usually several days after hatching before the first meal is taken.

#### *Technique.*

The parts were studied in serial sections, made at intervals varying from five minutes to three days after feeding. In making the dissections great care is necessary to avoid traction, which distorts the parts and disturbs the relation between the secreting cells and the contents of the gut in the early stages of digestion. For the study of the very early stages dissection was completed in the fixative, which enabled one to reduce the period intervening between the ingestion of blood and the fixation of the tissues, and at the same time rendered it easier to avoid rupturing the very delicate wall of the distended stomach. It is by no means easy to obtain satisfactory preparations of the early stages, when the stomach is still full of fresh blood. The periods during which the organ is immersed in the various reagents must be judged to a nicety, to ensure thorough penetration of the mass of blood and yet avoid injuring



the cellular wall. For clearing, cedar oil is the only satisfactory medium, and mixtures of cedar oil and paraffin of graded strengths must be used in order to reduce as far as possible the time necessary in the final paraffin bath. The sections were stained with Heidenhain's iron-alum-hæmatoxylin and counter-stained with eosin.

### *The Structure of the Mid-Intestine.*

The wall of the mid-intestine consists essentially of a single layer of secreting cells, which rest upon a basement membrane. The whole organ is surrounded by a double layer of longitudinal and circular muscle fibres. The cells are typically columnar in the resting condition, but their height and mode of arrangement differ according to their situation, and they undergo marked alterations in shape and structure during functional activity.

In the cardia the layer of cells is folded into innumerable small gland-like cæca, which project on the outer surface of the organ and give to it the mammillated appearance already noted (Plate LXI, figure 4). The lateral expansions are hollow structures, the lumen of which is in continuity with that of the central part (Plate LXI, figure 2). In the resting condition the walls of the cæcum are in contact with one another, but the position of the cells is not constant, and the potential lumen can be opened out by the pressure of fluid within the organ. The cæca are little more than short evaginations of the wall.

Cæca opening into the anterior part of the mid-intestine are found in many insects, both in the larval and adult stages, and are frequently long tubular structures of a permanent nature. Reduced structures representing cæca have been noted by Nuttall and Shipley in the mesquito (*Anopheles maculipennis*).

In the stomach the layer of cells is heaped up and compressed into a large number of small villi, which project into the lumen of the chamber and give to it, when empty, a stellate appearance in transverse sections. The basement membrane on which the cells rest is of course carried in with them, each villus having therefore the appearance of an invagination. As in the case of the cardia, the position of the cells is not permanent and one of the first results of the ingestion of food is the flattening out, and ultimate disappearance of the villi.

The muscles which surround the mid-intestine are arranged in two layers, an outer one of longitudinal fibres and an inner one of circular muscles, the latter being in close contact with the basement membrane.



Throughout the organ the muscles form a network which adapts itself closely to the irregularities of the surface, enclosing the protuberances of the cæca in its meshes and dipping into the hollows at the bases of the villi. At certain points, namely, at the junction of the oesophagus with the cardia, at the lower end of the cardia, and at the junction of the mid-intestine with the hind-gut, the circular fibres are strongly developed, and serve as sphincters to control the movements of the contents of the gut.

*The Epithelium in the Resting Condition.*

*The Cardia.*—The epithelium of the cardia (Plate LXI, figures 5 and 6) is columnar and regular. The cell membrane separating adjacent cells is never well marked, and may, in the parts of the wall between the cæca, be indistinguishable. The cytoplasm is dense, and shows a fine reticulum, the most conspicuous of the fibres of which run in the long axis of the cell. Vacuoles are rare, and when present are small and are situated in the inner half of the cell. At the inner border of the cell there is a remarkably well developed intima, which may have a thickness equal to one-eighth the length of the cell. It stains deeply with eosin, and shows up in sections as a ribbon-like border to the lumen of the organ, dipping into the cæca. Usually it has a homogeneous appearance, but under specially favourable conditions, as in very thin sections made from flies which have not fed for some time, a delicate longitudinal striation can be made out. The nucleus is round or oval, and contains numerous rounded granules of chromatin, for the most part arranged at the periphery. Mitotic figures are occasionally seen.

In addition to the columnar cells, small basal cells, which by their subsequent growth serve for the regeneration of the epithelium, are found here and there at the basement membrane, wedged in between the bases of the columnar cells. The majority of these are very small, and appear only as minute particles of chromatin lying in clearer areas of the cytoplasm, not definitely separated off from the larger cells (Plate LXI, figure 5). Cells intermediate between the basal and the columnar ones are frequently seen, pushing their way up towards the lumen (Plate LXI, figure 6).

*The Stomach.*—The columnar cells of the stomach (Plate LXIII, figure 18) vary in shape according to their position in the villus. The majority are long and conical, attached by their narrow ends to the basement membrane, and expanded towards the free border. At the

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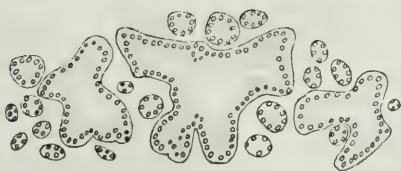


FIG. 2.



FIG. 3.



FIG. 8.

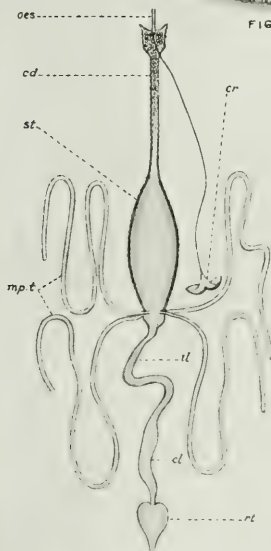


FIG. 1.

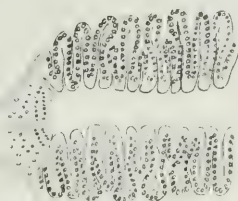


FIG. 4.

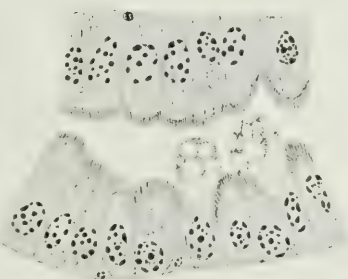


FIG. 5.

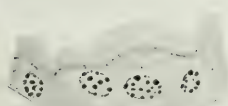


FIG. 7.

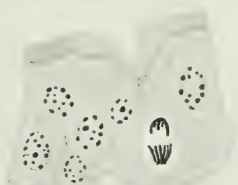


FIG. 6.



sides of the villus and between adjacent villi they are more regularly columnar. The cytoplasm is dense, and may contain one or two vacuoles of considerable size between the nucleus and the free border. The reticulum is closer, and the cytoplasm stains more deeply, in the part of the cell between the nucleus and the basement membrane, than in the inner half of the cell, except for the small area immediately below the intima, which stains very deeply with hæmatoxylin. The intima is delicate and shows a clear longitudinal striation. The nucleus is round or oval, and is situated towards the expanded end of the cell. In many of the cells small secondary nuclei are present between the large nucleus and the basement membrane. They appear to lie within the columnar cells without any separation of the cytoplasm. The basement membrane on which the cells rest is stout and conspicuous.

#### *The Epithelium during Functional Activity.*

##### A. THE STOMACH.

The most striking changes during the functional activity of the cells are seen in the stomach, and it will be convenient to consider this part of the organ first. As has already been pointed out, it is the stomach which receives the blood ingested during a meal; the cardia does not become distended, and the hind-gut is shut off by the closure of the sphincter muscle between it and the mid-intestine. In the stomach the blood is subjected to the action of ferments, and the assimilable substances produced are absorbed by the epithelium.

As the blood passes into the stomach the villi are flattened out, and as the distension proceeds the high columnar cells are stretched out by the increasing pressure of blood, until finally they become reduced to an even layer of cells more nearly resembling a pavement epithelium (Plate LXII, figures 9 and 11). In sections of the organ fixed an hour after a meal the whole of the epithelium of the stomach is found to have been reduced in this manner. A similar reduction of the columnar epithelium to flat cells has been noted in the mosquito by several observers.

During the remaining period, up to the full regeneration of the epithelium in preparation for the next meal, one finds no cells resembling those described and figured by van Gehuchten and other writers as secreting cells. Such cells are always numerous in the mid-intestine of *Musca* and other non-hæmatophagous flies, without regard to the period which has elapsed since the last meal, and their absence in *Tabanus* is

remarkable. It was the fact that they were not to be found as one expected which led to a closer examination of the mechanism of secretion in this genus.

Sections of the stomach prepared from flies killed and fixed within an hour of the meal usually show a few cells of the type described by van Gehuchten, especially towards the lower end of the organ. The shorter the time which has intervened between feeding and fixation, the more numerous these secreting cells are found to be. If special measures are taken to reduce the period between feeding and fixation to a minimum, the whole process of secretion can be followed. In the series of preparations from which most of the details given below have been worked out, the period was five minutes; the fly was allowed to take only about half the normal amount of blood.

Such a series of sections shows the whole mechanism of secretion, for the process does not take place simultaneously throughout the organ but begins in the upper part and extends downwards as the blood flows in. One finds, therefore, cells which have completely discharged their contents and others in which the process is just commencing in the same series of sections. The discharge of the secretion is in fact simultaneous with the flattening of the villi and the stretching of the columnar cells to a flattened epithelium. When the fly is undisturbed and takes a full meal under normal conditions, practically the whole of the cells have discharged their secretion and become flattened out by the time the ingestion of the blood is completed. Occasionally a few cells at the lower end of the organ retain their secretion for a short time afterwards, but one very rarely finds secreting cells later than an hour after the meal.

The processes to which the entry of the blood into the stomach gives rise are striking and rapid. In the hungry fly the cytoplasm of the epithelial cells (Plate LXIII, figure 18) is compact, containing at most one or two large vacuoles and frequently none at all. In the newly fed but not fully gorged fly, on the other hand, the least altered cells, though still columnar in shape and arranged in villi, contain large numbers of vacuoles in the part between the nucleus and the inner border; these vacuoles increase in size and tend to coalesce towards the intima, almost entirely replacing the cytoplasm. The delicate striations of the intima are lost, and the structure is replaced by a homogeneous layer of slightly greater thickness. The vacuoles, which contain the secretion of the cell, are collected into a mass at the free end as the intima

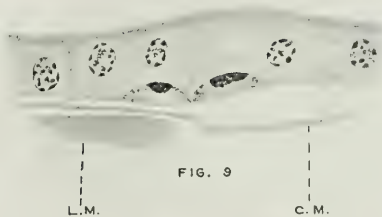


FIG. 9

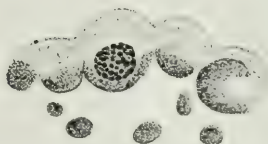


FIG. 10

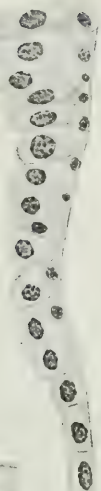


FIG. 12

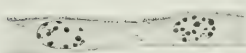


FIG. 11

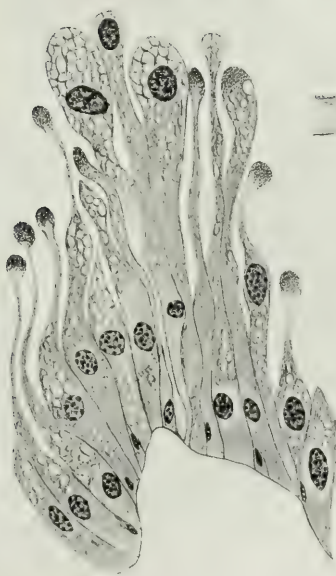


FIG. 13



FIG. 14





disappears. The mass of globules then passes towards the lumen of the stomach, that part of the cell between it and the nucleus being drawn out into a long neck (Plate LXII, figure 13), which ruptures, setting free the globules of secretion. When this has occurred, the cell again resumes its columnar shape by the withdrawal of the extended neck, and the intima is re-formed.

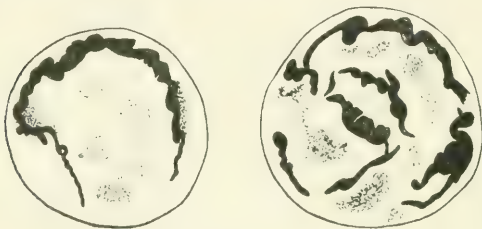
This casting off of the inner part of the cell with its contained globules of secretion frequently occurs simultaneously in several adjacent cells, with the result that a row of masses of globules is formed parallel with the cells (Plate LXII, figure 10). In a certain proportion of the cells the nucleus is included in the portion which is thrown off. Such nuclei always show, by their increased size and diffuse staining, evidences of degeneration. At this stage of secretion the epithelium of the stomach is separated from the ingested blood by a layer of spherical masses of globules, together with many degenerating nuclei, each of the latter being surrounded by a layer of highly vacuolated cytoplasm.

The columnar cells are reduced in size by reason of the discharge of their secretion. As the distension of the organ proceeds, they lose their columnar shape and become cubical; after a full meal under natural conditions they are still further stretched until the thickness of the cell is little, if at all, greater than the short diameter of the nucleus (Plate LXII, figure 11). The thickness of the cells varies at different parts of the wall in each series of preparations, since it depends to a certain extent upon the stretching of one part and the relaxation of another during the peristaltic contractions of the muscles surrounding the organ.

The digestive substance discharged by the cells is presumably in a fluid state, and the appearance of the masses in preparations is due largely to the precipitation and coagulation resulting from the reagents used. The masses are spherical or oval, and measure from four to twenty-four microns in diameter. Some of them are entirely composed of minute granules which stain deeply with hæmatoxylin. Others consist of large vacuoles in a matrix of granules (Plate LXII, figure 14). These masses of secretion are soon broken up, and by the time the epithelium is fully stretched they can no longer be distinguished. Degenerate nuclei, however, can be recognised up to a late stage.

One of the results of the action of this digestive substance on the red corpuscles is the formation of pigment. This occurs where the layer of globules comes into contact with the blood contained in the lumen of the stomach (Plate LXII, figure 14). The pigment is at first in

small scattered granules of a dense black colour, but later, as a result of the peristaltic movements of the wall of the stomach, which are going on as the ingestion of blood proceeds, it becomes collected into a densely packed layer which runs around the outer part of the lumen. As is shown in the text-figure, this layer has a very irregular outline, and shows indentations which are the impress of the villi. The effect of the peristaltic contractions of the wall is to drive the pigment—presumably the heaviest part of the stomach contents—towards the middle and



Transverse sections of the stomach of *Tabanus*, immediately after feeding, showing the arrangement of the newly formed pigment. From the same series of sections.

lowest part of the lumen, and here it is found collected in a dense mass in some preparations. The pigment does not pass into the hind-gut at this stage of digestion.

In addition to the above process of secretion, in which a large bulk of digestive fluid is thrown off in one act, and which takes place only at a special phase, there is another method, in which small quantities of fluid are discharged over a much more extended period. At a period subsequent to the re-formation of the columnar epithelium, and before the stomach is completely emptied, many of the cells show at their border minute spherical droplets, which appear to pass through the striated intima, pushing their way between the striæ, the latter closing up as the droplet emerges (Plate LXI, figure 8). Some of these droplets, fixed in the act of leaving the intima, are compressed by the striæ in such a way as to give them a flask-like shape. The droplets are always minute and clear, showing none of the deeply staining granules which are conspicuous in the large masses of globules described above. Cells showing this method of secretion are specially numerous in preparations made from

flies one day after a meal of blood. Once the droplets have left the intima they cannot be traced, for they are much the same size and shape as the shrunken remains of the red corpuscles.

## B. THE CARDIA.

The cells of the cardia also yield a secretion which is discharged into the lumen and is mingled with the ingested blood. The discharge of the secretion, however, does not take place suddenly at the time of feeding, but is continuous during the period of digestion, and takes place even when the stomach is empty.

Secreting cells (Plate LXI, figure 5) resemble those described in the mid-intestine of other insects more closely than do those of the stomach. The thick intima disappears, and the inner part of the cell is converted into a mass of large vacuoles, the matrix between which contains many deeply staining granules. The granules are much less numerous and much finer than those in the secreting cells of the stomach, and only make up a small portion of the mass, so that the whole has a more delicate appearance. The mass projects into the lumen and becomes rounded off and separated as a whole from the cell. Several such masses, all of approximately the same size and of a uniform texture, are often found in one cæcum, compressed between the opposing layers of cells or in the act of passing out of the cæcum into the lumen of the cardia. The mass of secretion, however, is never sufficient to distend the lumen of the cæcum. Cells in the act of secretion are found side by side with cells which show no signs of activity.

The cells of the cardia are evidently not dependent on the stimulus of ingested blood in order to discharge their secretion. Secreting cells have been found in bred flies fixed three days after hatching, while still unfed, and in others three days after a full meal. They are most numerous, in fact, in hungry flies, at a time when the stomach is empty.

As the spherical masses of secretion pass out of the cæca into the lumen of the cardia they are broken up, and reduced to a granular debris which takes on the eosin stain strongly. During the first few hours after the meal the lumen frequently contains large amounts of this substance, intermingled, as will be explained later, with the red corpuscles which are found in the cardia at that period.

Secreting cells of the second type, in which small clear droplets of an unstainable substance emerge between the striæ of the intima, are occasionally seen in the epithelium of the cardia.

*Regeneration of the Epithelium.*

*The Cardia.*—Degenerate cells, the nuclei of which are swollen and retain the hamatoxylin stain deeply and diffusely, are frequently seen free in the lumen of the cardia. They are always in much smaller numbers than the secreting cells, which suggests that each cell may discharge secretion on several occasions before it degenerates. Nuclei are never included in the mass of secretion thrown off, as is the case in the stomach. The cells lost are replaced by the upward growth of the basal cells, which push their way towards the lumen. The supply of basal cells is kept up by the division of the nuclei of the columnar cells; the daughter nucleus which lies nearest the basement membrane in such a division, and which is at first equal in size to the other, becomes reduced, and takes to itself a small portion of the cytoplasm of the parent cell.

*The Stomach.*—The re-formation of the columnar epithelium from the stretched and flattened cells begins soon after the ingestion of blood has ceased. The cells lost are replaced by the growth of the basal cells, and as absorption proceeds and the stomach is gradually emptied of its contents the cells are re-grouped into villi. New basal cells are formed by the division of the nuclei of the columnar cells.

The early stages can be most easily followed in preparations made from flies which were not allowed to finish the meal, as in these both the fully stretched cells and the early stages of the re-formation of the villi can be seen. In portions of the epithelium which are stretched to the maximum the basal nuclei are seen lying against the basement membrane, and have the same appearance as those in the columnar cells in the resting condition. As the tension relaxes and the cells become cubical in shape, growth of the basal cells begins. In parts of the epithelium which have been fully stretched, the enlargement of these basal cells is mainly in the diameter parallel with the basement membrane (Plate LXII, figure 9), and the growing cell has an oval shape, flattened towards the basement membrane, with the nucleus lying on the side towards the intima and near the periphery. Eventually these cells reach the inner border of the epithelium and acquire a striated intima.

Within four hours of the meal, the epithelium consists of a regular row of columnar cells (Plate LXIII, figure 15). It is at this stage that the new generation of basal cells is produced. The nuclei, which in the resting epithelium are very uniform in size and structure, now show a considerable range of variation. Many are larger than those of the resting epithelium, and all sizes from these large nuclei down to those of normal



FIG. 15



FIG. 16



FIG. 17

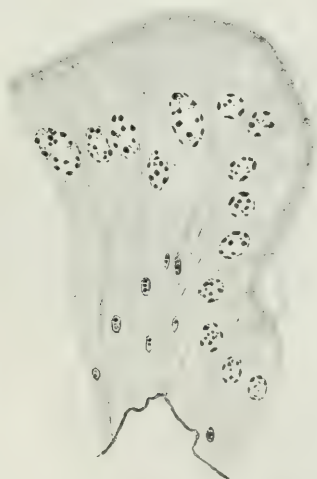


FIG. 18



basal cells are found. The majority are round or regularly oval, but many of the oval ones are pointed and irregular at the end which is directed towards the basement membrane. There is in almost all the nuclei a large and deeply staining nucleolus, which may be round, oval, or rod-like; in some nuclei it has a comma-shape, as if a small portion of the chromatin were being thrust out. Many of the nuclei are elongated, and in some a constriction is present separating the nucleus into two unequal parts, of which the smaller is in the basal part of the cell. Though it is evident that the nuclei are dividing, mitotic figures have not been found. The chromatin is arranged in large granules and rods of irregular size and shape. It appears as if the basal nuclei are formed by the unequal and amitotic division of the principal nuclei.

At this stage the cytoplasm shows a very fine reticulum, with no vacuoles. As the growth of the cell continues, it takes on an alveolar structure, while the basal portion of the cell becomes considerably denser than the rest. The principal nuclei remain about the middle of the cell as its height increases, and nucleoli are no longer distinguishable; the basal nuclei remain near the basement membrane, and gradually diminish in size. At the end of eight hours (Plate LXIII, figure 15), there are already indications of the formation of villi.

At the end of twenty-four hours the villi are well formed. The principal nuclei are now arranged in a regular row about the middle of the cell, and the basal cells are small and usually elongated. The cytoplasm is clearly differentiated into two parts: internal to the nucleus it consists of an open network enclosing large alveolar spaces, while between the nucleus and the basement membrane it is dense and shows only a few laterally compressed alveoli. By the end of the second day the villi are still further increased in height, and project into the mass of finely granular eosinophil debris which is now all that is left of the blood. The cytoplasm is denser, the alveoli smaller. After three days the villi have reached their full height and give to the lumen of the stomach a stellate appearance in transverse sections. The cytoplasm is now dense throughout the cell, and one or two large vacuoles may be present between the intima and the nucleus. The basal nuclei lie in the narrow attached end of the cell, a little distance from the basement membrane.

#### *Absorption.*

Digestion and absorption of the ingested blood proceed *pari passu* with the re-growth of the epithelium. In sections of the recently fed



fly the red corpuscles are found to have their normal size and shape, but within a short time they become distorted and reduced in size, and also lose their affinity for the eosin stain. The envelopes of the red cells remain as 'shadow cells' for at least twenty-four hours, intermingled with a granular material, the product of the disintegration of the masses of secretion, which takes on the eosin stain well. Pigment is formed at a very early stage, and by the end of two hours the contents of the stomach have a deep purple colour, which subsequently darkens as the contents are reduced to a tarry mass. As has already been stated, *Tabanus*, like most blood-sucking Diptera, has the habit of defæcating during or shortly after feeding, and in the recently fed fly the hind-gut is therefore empty. The sphincter between the mid-intestine and the hind-gut remains closed during the early stages of digestion, and it is not until eight hours or more that any of the contents of the stomach are allowed to pass. After this period a brownish black residue passes into the ileum and eventually accumulates in the rectum, which usually contains a little of this substance at the end of the first day. In two days the stomach is empty, or contains at the most a little granular matter and traces of a black pigment derived from the blood.

#### *Peristaltic Movements. Function of the Crop.*

It has already been pointed out that during the ingestion of blood the wall of the mid-intestine undergoes peristaltic movements, brought about by the alternate contraction of the longitudinal and circular muscles. The effect of these is to bring about an intimate mixture of the digestive substances with the blood; at a very early stage the masses of globules thrown off by the cells are broken up, and reduced to a finely granular material which takes on the eosin stain. At first this material appears as a dense band, often loaded with coarser pigment granules of an intense black colour, lying between the still intact globules and the unaltered cells (Plate LXII, figure 14), but within half an hour of the meal it is intimately mixed with the mass of corpuscles.

These peristaltic contractions are continued after completion of the meal, and are not limited to the stomach, but extend also to the cardia and the crop. Though the cardia is never greatly distended with blood, during the first few hours after the ingestion of the blood its lumen is usually found to be opened up and to contain red corpuscles, together with an eosin-stained granular material similar to that found in the stomach. In many parts the cæca are opened out so as to bring the cells in their walls

into contact with the contents of the lumen ; red corpuscles are frequently found in the lumen of the lateral prolongations at the anterior end of the cardia. The degree of dilatation varies in different preparations, but is never more than is sufficient fully to open the mouths of the cæca, giving them a horse-shoe-shaped outline. The effect of the extension of the peristaltic movements to the cardia is to secure a thorough mixing of the secretion discharged from its cells, and accumulated in the cæca, with the contents of the stomach.

The crop is never found to be distended in flies dissected immediately after feeding, and it does not, as in the mosquito, serve as a food reservoir. Up to an hour or more after the meal, however, it is always found to show a slow regular pulsation, and frequently contains a trace of blood. In some dissections it is found to contain a clear yellowish fluid. It would appear that during the peristaltic movements some of the contents of the mid-intestine are forced back into the crop, and again returned by the latter—a process which would assist in bringing the secretion of the cardia into intimate mixture with the ingested blood.

#### *Summary and Conclusion.*

The processes of secretion and epithelial regeneration in the mid-intestine of *Tabanus* may be summed up briefly as follows: The whole of the epithelium consists of secreting cells, divided into two groups, those of the cardia and those of the stomach, which function differently. The cells of the cardia continue to discharge their secretion at all stages of digestion, though only a small proportion of the epithelium is secreting at any one time. The cells of the stomach, which is the part in which the blood is retained during digestion, throw off their secretion only at the time of feeding, while they are being stretched out from a high columnar epithelium arranged in villi to a row of flattened cells. In each case the secretion is thrown off from the cells as a mass of globules, which are subsequently broken up, the contents appearing in sections as a granular eosinophil substance. The process of digestion occupies about eight hours, during which period the mid-intestine is shut off from the hind-gut by the closure of the sphincter muscle at the junction of the two parts. The secretion of the cells is intimately mixed with the blood by means of peristaltic contractions of the wall of the mid-intestine. The absorption of the products of digestion is carried out by the stomach, the cells of the epithelium of which have a loose alveolar cytoplasm while absorption is taking place. The regeneration of the epithelium is brought

about by the upward growth of basal cells which are present in the resting epithelium. In the stomach these cells grow towards the lumen soon after the meal, and new basal cells are formed by the division of the nuclei of the larger cells within about four hours. As absorption proceeds and the lumen of the stomach empties, the epithelium is again grouped into villi, which are complete at the end of three days.

The great thickness and unusual staining reactions of the intima of the cardia are noteworthy features. Its appearance led the present writer, in a previous publication, into the error of describing this part of the mid-intestine as a part of the fore-gut. The crop, or œsophageal diverticulum, does not function as a food reservoir. There is no peritropic membrane. As regards absorption of the digested food, this passes through the cytoplasm of the cells of the stomach without assuming a concrete form, and there is nothing resembling the complex phenomena which have been described in connection with absorption in the Ticks.

In view of the close relation of the two families, and the general similarity in their structure and habits, it is probable that processes similar to those described in *Tabanus* also occur in the mosquito.

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## THE ROYAL AIR FORCE MEMORIAL FUND APPEAL.

PATRON :—H. R. H. PRINCE ALBERT.

CHAIRMAN :—THE RT. HON. LORD HUGH CECIL,

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*19th January, 1920.*

TO THE EDITOR,

*Indian Journal of Medical Research.*

SIR,

WE should be very glad if you would afford us the hospitality of your columns to make an appeal on behalf of the Royal Air Force Memorial Fund.

This Fund has been established to commemorate the services of the Royal Naval Air Service, the Royal Flying Corps, the Australian Flying Corps and the Royal Air Force during the war, by an organisation which will secure such lasting benefits to the Officers and Men of the Royal Air Force, and their dependents as may be worthy of the greatness of the achievements commemorated.

The Executive Committee of the Royal Air Force Memorial Fund while taking care that their Memorial should distinctly commemorate the Royal Air Force have equally been anxious to avoid mischievous overlapping, and have with that purpose put themselves into communication with the United Services Fund, with Lord Haig's Central Committee and with the Flying Services Fund.

The objects the Executive Committee have decided to pursue are :—

The erection of a commemorative monument to the fame of the  
Royal Naval Air Service, the Royal Flying Corps, and the

Royal Air Force including the officers and men who joined the Force from Canada, New Zealand, South Africa and the other over-seas Dominions.

The establishment of places of residential education (like Trafalgar Homes) for the children of Airmen.

The provision of bursaries available at approved schools.

Generally the provision of such treatment and the rendering of such assistance, as means may permit either directly or in co-operation with other organisations, to Officers and Men and their dependents who may be disabled, sick, or otherwise infirm.

All officers and men of the Flying Services, whether from the Dominions or from the United Kingdom, will, of course, be eligible for these benefits.

These objects will be furthered by the Royal Air Force Memorial Fund in the closest co-operation with the United Services Fund and with Lord Haig's Committee in accordance with the requirements of each particular object so as to prevent overlapping in expenditure; and the Executive Committee are confident that they will both fittingly commemorate the precious national services of the Royal Air Force and realise with due economy the benefits for those whom it is sought to help. An approximate estimate shows that a large sum, probably £ 400,000, will be required. It is necessary, therefore, to appeal to the abundant generosity of those who honour the memory of the services and sufferings of the Royal Naval Air Service, Royal Flying Corps, the Australian Flying Corps and the Royal Air Force during the war.

We know that in many hearts the memory of these services glows unforgettable. To some it is intertwined with the agony of bereavement; to some it speaks of happy friendship and pleasant reminiscence; but by all who endured the anxieties and rejoiced in the glory of the Great War, not the least honoured place in the proud and thankful recollection of its chequered days is given to the skill and nerve of the brave men who first made war in the unbounded arena of the air, and to the ingenuity and industry of those who rendered that gallant fighting so fruitful to the cause of victory. To all in whose minds these memories are enshrined we now appeal; of everyone whose heart quickens with pride or pain when he recalls the warfare in the air we ask that these sentiments of patriotism and of affection shall now be shown in a liberality not unworthy of their high temper, and that he will join with us in raising

a lasting Memorial which shall carry down to a remote posterity the shining tradition of the Royal Air Force in the War, of its fine courage and its great renown.

Inquiries, Donations and Subscriptions should be addressed to :—

DEREK McCULLOCH, Esq.,

Secretary,

25, *Victoria Street,*

*Westminster, S.W.1,*

*London.*

(Sd.) ALBERT.

HUGH CECIL.

HUGH M. TRENCHARD.

J. M. SALMOND.

A. V. VYVYAN.



## EDITORIAL NOTES.

THE Editors direct the attention of Indian research workers to the conditions attached to the Dr. A. Mitra Memorial Research Scholarship. Further particulars can be obtained on application to the Honorary Secretary, School of Tropical Medicine, Calcutta.

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### CALCUTTA SCHOOL OF TROPICAL MEDICINE.

#### THE DR. A. MITRA MEMORIAL RESEARCH SCHOLARSHIP.

The Dr. A. Mitra Memorial Research Scholarship has been endowed in memory of the late Dr. A. Mitra of Kashmir by his widow and will be awarded under the following conditions :—

1. The scholarship shall be open to a pure native of India, including the State of Kashmir.

2. The candidate must be a registered medical graduate or licentiate and must have already worked for some time in a scientific laboratory and have shewn aptitude for, or proficiency in, research work.

3. The subjects of investigation shall be the diseases of metabolism and during the first four years the research shall be in connection with diabetes.

4. The scholarship shall be for four years. The holder shall report the results of his work to the Committee annually, or as called upon to do so.

5. The research scholar may be allowed consultant, but not general, private practice outside his laboratory hours.

6. The research scholar, in the first instance after appointment, shall be directed to proceed to some laboratory in Europe or America, as selected by the Committee, for a year's training. An allowance of Rs. 1,000 for outfit and return passage will be granted. The scholar shall receive not more than Rs. 250 p.m. whilst in Europe or America. On return to India the value of the scholarship shall be Rs. 200 p.m. whilst working in India.

7. Applications for this scholarship should reach the Hony. Secretary, Calcutta School of Tropical Medicine Endowment Fund, not later than the 31st August, 1920, and should be supported by certified copies or originals of testimonials, etc.



## A NOTE ON THE BEST METHOD OF OBTAINING PRECIPITATING ANTISERA.

BY

LT.-COL. W. D. SUTHERLAND, C.I.E., M.D.\*

AND

ASST.-SURGEON RAI G. C. MITRA BHADUR, L.M.S.

[Received for publication, May 15, 1920.]

FOR some years we have been experimenting here with a view to ascertain which method of injection of antigen is the best for securing a perfectly specific precipitating antiserum of high potency. We have not yet satisfactorily solved the problem, but hope to do so some day. We have used the intraperitoneal and intravenous methods of injection, alone, and in various combinations. We have given various doses at various intervals, and we have bled the fowls so treated at different times after the last injection of antigen. Briefly our experiments may be summarised thus :—

- |      |   |              |
|------|---|--------------|
| I.   | 4 doses of antigen, intravenous, intervals of 3 days between doses. |              |
|      | doses 2—3c.c.   |              |
|      | „ 4—4.5c.c.   |              |
| II.  | 3 doses. A 3 intravenous, at 3-day intervals                        | 4—5 c.c.     |
|      | „ B 2 intravenous, do.  | 4—5 c.c.     |
|      | „ 1 intraperitoneal, after 7 days,                                  | 10 c.c.      |
|      | „ C 2 intravenous, 3-day intervals, 5 and 8 c.c.                    |              |
|      | „ 1 intraperitoneal, after 4 days,                                  | 10 c.c.      |
|      | „ D 2 intravenous, 3-day intervals, 5 and 10 c.c.                   |              |
|      | „ 1 intraperitoneal, after 4 days,                                  | 10 c.c.      |
| III. | 2 doses. Intravenous, 3-day intervals,                              | 4 and 8 c.c. |

After prolonged trial the intraperitoneal injections were discontinued, because they had the disadvantage of consuming a large quantity of

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\* The Editorial Committee record their sense of the great loss suffered by medical research in India by the death of Lieutenant-Colonel W. D. Sutherland, C.I.E., I.M.S., who died at Calcutta on the 27th June 1920.

antigen, without any compensating advantage of being more productive of antibody than intravenous injections.

The two-dose method is easier to carry out, and consumes less antigen than the three-dose method while it gives practically the same results. Therefore it is to be recommended. On the whole we find that it is best to bleed the animal on the fourteenth day after it has received the last injection of antigen.

Of 1,613 fowls treated 296 (18·35%) died, mostly of pasteurella infection; 665 (41·23%) did not furnish a useful antiserum, and from 652 (40·42%) we obtained a good and specific antiserum.

As we are now experimenting with prophylactic vaccination of our fowls against pasteurella, it is probable that we shall have better results in future. Even as they are, our results are not so bad when compared with those obtained elsewhere under conditions much more favourable to animal life than the hot, very moist and excessively bacteria-laden atmosphere of Calcutta.

# TECHNIQUE OF AGGLUTINATION.

BY

LIEUT.-COLONEL W. F. HARVEY, M.A., M.B., D.P.H., I.M.S.,

*Director, Central Research Institute, Kasauli.*

[Received for publication, May 3, 1920.]

THE consideration of the technique of agglutination involves the discussion of titre, and of method.

## I. *Titre.*

By 'titre' is meant that value which represents the potency of a serum and which is descriptive of the results of its action. It is not too much to say that there is no consensus of opinion as to how this value should be expressed. Great advance has undoubtedly been made in precision with the adoption of Dreyer's method, in so far as it involves standardization of reagents. I may, by quotation, illustrate the statement, that no consensus of opinion exists on what is to be taken as the 'titre' of a serum :—

1. The titre of an agglutinating serum (cholera) is that quantity of serum which just suffices in 1 hour at 37°C to cause the appearance of flocculi visible to the naked eye in 1 c.c. of a suspension of 1 loopful of an 18-hour virulent culture in 0.8 per cent salt solution. Kolle and Schurmann in *Handbuch der path. Mikr.* Vol. IV, p. 66, Kolle and Wassermann, 2nd Edition.

2. (a) 'Standard' agglutination is the degree of agglutination present in the highest serum dilution in which marked agglutination without sedimentation can be seen by the naked eye.

- (b) The 'standard' agglutinin unit is that amount of agglutinating serum which, when made up to 1 c.c. volume with normal saline solution, causes 'standard' agglutination on being mixed with 1.5 c.c. of the original standard agglutinable culture and maintained at 55°C for 2 hours (in the case of dysentery agglutination 4½ hours) in a water bath followed by 15–20 minutes at room temperature.
- (c) Total agglutination indicates the condition in which the whole or practically the whole of the agglutinated bacteria have settled down at the foot of the tube.
- (d) Trace of agglutination = a very fine granulation recognizable by the naked eye. Dreyer and Inman, *Lancet*, 10-3-17, p. 365.

3. The end point of agglutination is given by the highest dilution in which the cocci have clumped and sedimented, leaving the supernatant fluid clear or with the faintest suspicion of turbidity. When the turbidity, though slight, is distinct and agglutination well marked, the results may be expressed by the symbols  $\pm$  or  $\mp$ , the former meaning that agglutination is well marked but incomplete, the latter that agglutination is slight but definite. The 'cocci' referred to are meningococci. Griffith, *Rep. L. G. B.* 110, 1916, p. 43.

4. The macroscopic titre published with the sera distributed indicates the highest dilution of the serum giving an agglutination which is readily seen with the naked eye under the stated conditions. The conditions are:—(1) The use of suspensions in distilled water of living 20 hours agar cultures; (2) the use of suspensions standardized by opacity; (3) the agglutination tubes are placed in the incubator for 2 hours, or 4 hours, with 20 minutes at room temperature before reading results. The tubes are left overnight at room temperature and read again for confirmation of the first reading. Chick, *Lancet*, 22-4-16, p. 858.

5. When the end point of the reaction is to be read off we must institute an arbitrary time limit; a limit of 24 hours will be found convenient. After the lapse of that time we read off the highest dilution in which we have a complete, or if we prefer to make this our criterion an incomplete, sedimentation reaction. Sir A. E. Wright. *Technique of the Test and Capillary Tube*, p. 105.

6. If there is agglutination, there form at first light particles; then after 20 or 24 hours true flocculi deposit at the bottom of the tube whilst

the supernatant fluid clears partially or wholly. The particles are of various sizes. They can be very fine and difficult to see with the naked eye. They should then be examined microscopically. Macé. Bacteriology, Vol. 1, p. 409.

7. Any definite agglutination which can be detected with a hand lens may be taken as positive. Fildes and Baker. Med. Res. Comm. Report 17, 1918, p. 81.

The fact of the matter is that agglutinating sera may give very finely graded results both in the same dilution and in successive dilutions. Any attempt to describe a supposed single end point or standard is bound to be highly artificial. Such end point or standard is dependent for its limit on the fineness of the gradation of dilutions of serum used and the amount of agglutination which may be taken as visible to the naked eye, or to the eye aided by a hand lens. This brings me to my proposition which is, that it is not desirable to seek an end point: rather should we endeavour to describe, minutely *and in full series*, the appearances obtained with standard reagents.

The basis of description of the phenomena of agglutination which I have adopted is a two-fold one. I use, and propose for use:—

(1) A description of the degree of turbidity (to the naked eye) of suspensions, after subjection for the allotted period to the action of an agglutinating serum in a series of dilutions. Under this description

*a*=turbidity unaffected, identical with that of control.

*b*=diminished turbidity as compared with control.

*c*=a mere residual opalescence. ~

*d*=complete absence of turbidity, with a water-clear supernatant fluid.

These may seem to be very loose descriptive terms but their use will become evident when they are taken in conjunction with the 2nd character which is degree of agglomeration of granules or flocculi produced by agglutination. The turbidity shows in a general way not only what is the size of the particles in the suspension but also the relative preponderance of fine or coarse particles. Thus with an 'a' turbidity the further description may show the presence of flocculi. But we should infer in that case that the flocculi are few in number or small in size, not to have produced any apparent change in the turbidity. The 'b' turbidity which corresponds in general terms to definite agglutination visible to the naked eye is descriptive of a somewhat wide range of effect and requires the qualification brought by the second character to



make it intelligible in a quantitative sense. At the same time the limits of 'b' agglutination are more or less those of the titre values in current use which do not adopt as their end point complete subsidence of bacteria. The 'c' turbidity is the common 'complete agglutination,' for it is seldom that a *trace* of opalescence cannot be detected in the supernatant fluid even with low dilutions of sera.

(2) The second character used in description is that given by the size of the agglomerated mass or masses of bacteria. This ranges from a compact single mass, through discrete masses and minute particles, down to what is merely incipient separation into particles thus :—

Stage 6 = that showing the presence of a compacted single mass of bacteria.

Stage 5 = that showing the presence of several compacted masses, differing usually from the former by having, on inspection, with the hand lens, a fringed or ragged character.

Stage 4 = that showing coarse particles or flakes.

Stage 3 = that showing fine particles.

Stage 2 = that showing very fine particles.

Stage 1 = that showing a ground glass appearance or appearance indicative of incipient breaking up into particles.

Stage 0 = that showing a uniform turbidity with total absence of agglutinative effect.

The appearance in the case of this 2nd character should be described, as demonstrated by the use of a hand lens of say 10 diameters magnification. In actual description one leaves out the use of the word 'stage' and runs the figures one into another thus :—

$120 = \frac{43210}{b}$  signifies that for a dilution of serum of 1-120 the appearance is that of a mixture of stages 4, 3, 2, 1 and 0 with a turbidity of type b.

There are many ways in which—if used—this method of record will contribute to the precision of record. Two tubules for example may show the presence of agglomerations of the same degree, such as 6, 5, 4, but if in the one case the turbidity is c and in the other b we infer that in the former the supernatant fluid or 'interfluid' is nearly clear and in the latter that it is not. The former will be the higher degree of agglutination. Again, suppose we have an agglutination  $\frac{65432}{b}$ , we should say that the compact plug of stage 6 is present and also the separate mass of stage 5, but that the suspension has nevertheless been only partially agglomerated.

As regards the precision of the nomenclature one has to admit that there will be variation. But if the whole range of results is given it is remarkable how near to uniformity of description one is able to get. Here are for example 2 tests done on 2 successive days in which the actual readings were done without knowledge in the case of the 2nd, of what they were on the previous day.

14-6-18. Para B.  $8=\frac{65}{c}$ ;  $512=\frac{65}{d}$ ;  $1024=\frac{5}{d}$ ;  $2048=\frac{5}{c}$ ;  $4096=\frac{54}{c}$ ;  $8192=\frac{54}{b}$

$$c^*=0$$

15-6-18.  $512=\frac{65}{d}$ ;  $1024=\frac{5}{d}$ ;  $2048=\frac{5}{c}$ ;  $4096=\frac{54}{c}$ ;  $8192=\frac{432}{b}$ ;  $c^*=0$ ;  $*c=\frac{0}{a}$  Control

tube containing 0.85 per cent salt solution instead of serum dilution.

In the *Indian Journal of Research*—Harvey (1916), p. 616—I described a method of agglutination reading on which the present system is based. In that paper I proposed that *time* should be taken as the measure of agglutination instead of *dilution*—time taken to reach a given stage of agglomeration with a given dilution of serum. The present method is in no way intended as a substitution method. At that time I was working with capillary tubes in which the stages—for the short periods of time used—were quite definite. That method probably gives as sharp effects as the one the details of which I am about to describe. But with the employment of tubules in place of capillary pipettes and a hand lens instead of merely naked eye observation it became obvious that one had to describe, in the tubules, a mixture of stages (or degrees of agglomeration) and not a single stage. The argument for the use of time as a measure of agglutination is not affected by the advocacy of the present mode of description. Time can be used as a measure, with this method, as with the previous one simply by (1) shortening the period of observation, (2) fixing the stage of agglomeration to be observed and (3) noting the dilution in which it is observed.

To summarize :—I contend that the titre of a serum is best expressed in terms of (1) the degree of flocculation produced, (2) the comparative turbidity of the suspensions used and (3) the dilution of the serum. These facts should be observed, if time is not taken as the actual measure, for a whole *series* of dilutions and not merely for a single final dilution. It is permissible of course to set forth the result in any form which will suffice to demonstrate the effect produced. A curtailment of the number of dilutions to be employed is merely a matter for agreement. If it is desirable that a single figure should be adopted for 'titre' record then

take either (a) the highest dilution of serum in which a degree of agglutination equal to stage 5 or 6, with turbidity c or d, has occurred or (b) the highest dilution of serum in which agglutination observable by a lens magnifying 10 diameters has occurred, always giving a description of the degree of agglutination obtained and requiring that the 'turbidity' should be at least of b grade.

## II. *Method.*

The methods employed for carrying out the agglutination test are very numerous. These involve the use of what may, for purposes of discussion, be regarded as variables in a mathematical function. Such variables are :—test receptacle, time, temperature, dilution of serum and of suspension, nature of the diluting and of the suspending fluid, etc. It may be of use shortly to consider how these may vary, the possible effect upon the result and some of the advantages and disadvantages of the variations.

*Test receptacles.* These may be capillary pipettes, glass tubules, or small test tubes. It is doubtful whether there is any great difference to be found in the results according as one or other of these is used. But in the tubule, and still more in the test tube, it is possible, to a greater extent than in the capillary tube, to observe with a hand lens the mixture of different degrees of agglomeration of the suspension brought about by agglutination. This may not be altogether an advantage, as it necessitates much greater minuteness of detail in the description of the effect. The tubule has the great advantage over the test tube—at least in the method of test which I am about to describe—of permitting of inversion for the purposes of obtaining a readable result. I consider inversion of tubules to be preferable to shaking or stirring the agglutinated deposit, as it is extraordinary how easily the deposited flocculi are broken up and brought again into homogenous suspension.

*Time.* Time as a variable is constantly used in conjunction with dilution. The commonly accepted method is it to vary the dilution and fix the time. But as I have shown (Harvey, 1916) it is perhaps more definite and capable of furnishing more delicate gradation of result to fix the dilution and vary the time. In the former case dilution is used as the measure of titre and in the latter time. The following table shows an experiment with *B. dysenteriae* (Flexner) designed to show how time may be used as the measure of agglutination.

TABLE.

*Showing stages of agglomeration of suspension with time,  
in water bath at 37° C.*

Dilution of Serum.	Minutes of observation at 37° C.										After 24 hrs. at room temperature.
	0	15	30	45	60	75	90	105	120		
1-20 . . . . .	0	2	2	3	3	4	4	5	5	5 d	
1-200 . . . . .	0	0	0	0	0	2	2	3	3	534 c]	
Control . . . . .	0	0	0	0	0	0	0	0	0	0/3	

*Magnification.* The extension of degree of visibility of agglutination with increasing dilutions of serum is a matter of the magnification used. Especially is this so if a description of degree of agglutination in detail is used. There is, however, no essential difference, other than this between agglutination as determined by a microscopic or a macroscopic method. A hand lens ( $\times 10$ ) is convenient to use for description involving gradation of agglomeration.

*Dilution of serum and of suspension.*—(a) *Serum.* For the most part dilutions of serum proceed by multiples of two. Some variation from this is introduced occasionally by altering the gradation at different points as *e.g.*, in such a series as 50, 100, 250, 500, 1000-fold dilution where an alteration to a multiple of  $2\frac{1}{2}$  occurs after 100 and again a restoration to the multiple of 2 thereafter. There does not seem to be anything to commend such a procedure. Dilution by multiples of two is a satisfactory method and is only liable to break down in the higher dilutions. A result which shows agglutination up to 250 and none in 500 tells us little of what the true value is, obviously a value between these two points. One method employed is to take the mean of the 2 values. With time as measure this difficulty does not arise nor does it truly arise when my presently advocated method is employed because with that method an endeavour is made to give an exact description of the degree or grade of agglutination attained with *each and every* dilution in fixed time. This method should afford a finer approximation to the stage reached than that which is given by the use of terms such as complete, partial, or trace.

(b) *Suspension.* There are undoubtedly differences observable in the result according to the dilution of the suspension. In my paper on 'Time as a measure of agglutination,' I recommended the use of a very concentrated suspension as tending to accelerate the result and give a definite reading. In the method now described I use a moderate concentration, equivalent to 1 mgm. of dried bacterial substance in 1 c.c. of diluent. Dreyer uses suspension of an extreme degree of tenuity. These latter give very clear results and show no tendency to spontaneous settlement, which are matters of great advantage. My own method was adopted mainly on account of the ease of standardization afforded by Brown and Kirwan's tables (1915). That or a similar method of standardization is capable of being applied in any laboratory, and gets over the disadvantage of being dependent on a central source for standard agglutinable suspensions. Whether a still greater degree of standardization is necessary, as would be implied in the use of one strain of organism only, grown on a standardized medium, for a fixed period of time, it would take time and close experiment to decide. That difficulty, if it is a real one, might be got over by supplying, along with agglutinating sera, a sample of the antigen used in evaluating its titre, for purposes of comparison with the laboratory antigen.

*Temperature.* The temperatures used by different observers, or combined in the course of test, are 50°-55°C, 37°C, and room temperature. A water bath at the given temperature is often used instead of the air incubator at 37°C. The temperature desired is much more rapidly taken up with the water bath and the method therefore is more standard, but the incubator, being always at hand, is more convenient.

*Dilution of serum and suspending fluid.* The fluid which is used for both is usually 0.85% salt solution and this is what I have used. I have, however, found considerable advantage in using distilled water both for dilution of the serum and for making the suspension. In the case of the serum a fine precipitation, as one would expect, is produced on the addition of distilled water, visible, however, only in the greater concentrations. In the case of the organism distilled water has the advantage over 0.85% salt solution occasionally when the strain of organism used exhibits a tendency to agglomerate spontaneously. My experience has not shown that there is any marked difference in the results obtained by the use of the one or the other. It is just possible that the semi-precipitation occurring in the serum with the addition of distilled water may render the test more delicate or more rapid especially if precipitation out of the

serum itself as well as flocculation of the bacteria are both factors in the agglutination process (cf. the relations precipitinogen, precipitin, and precipitum according to Welsh and Chapman). All the variables may be fixed at will—the only desideratum being that there should be some consensus of opinion on the subject of fixity. What is difficult to fix, however, is not discussed in connection with these variables and that is the description of the phenomenon itself. I have given what I think is a satisfactory description of this phenomenon. The following is the full detail of the suggested technique of the test.

*Technique of Test.* (1) Use 13 tubules<sup>1</sup> (4 cm. in length and 3 mm. internal diameter) set up in a row in a plasticine bed.<sup>2</sup> (2) Set out a porcelain slab with 12 depressions, a set of watch glasses, Wright capillary pipettes fitted with teats, a tin of boiling water with Bunsen flame beneath, platinum loop, grease pencil, opacity tubes and standard print,<sup>3</sup> hand lens ( $\times 10$ ) and flask of 0.85 per cent sterile salt solution (0.85 S.S.S.). (3) Take a 24-hour culture<sup>4</sup> of the test organism. (4) Mark the test tube with a grease pencil at a point approximately representing one-third of the culture surface.<sup>5</sup> Fill in 0.85 S.S.S. to this level. (5) Make a suspension of the growth in the 0.85 S.S.S. (6) Pour into a watch glass. (7) Take up unit volume of suspension with a capillary pipette having a mark on the stem, and place this volume in the opacity testing tube. (8) Add successive volumes of 0.85 S.S.S. (9) Make comparisons, after each addition over standard print with the standard opacity tubes (Brown and Kirwan, 1915) until the test tube matches with a standard tube. (10) Compute the strength of the suspension and the amount of dilution with salt solution which is required to make the suspension equivalent to 1 mgm. of dried bacterial substance in 1 c.c. fluid. (11) Make the dilution required, using the same capillary pipette with a suitable mark.<sup>6</sup> (12) Take up one volume of diluted suspension and an equal volume of salt solution. (13) Mix and place<sup>7</sup> in the 13th tubule (salt control). (14) Reject the capillary pipette used up to this point, and also the salt solution used. (15) Pour out fresh salt solution. (16) Take a new Wright capillary pipette and mark a point on the stem with the grease pencil, the unit-volume point. (17) Place one volume of salt solution in each of the 12 depressions of the porcelain slab. (18) Take up one volume of test serum and add to the salt solution in the first depression. (19) Mix and remove<sup>8</sup> one volume of the mixture. (20) Add the removed volume to the salt solution in the second depression. (21) Mix and remove the



volume of this mixture for addition to the salt solution in the third depression. (22) Continue the process to the 12th depression, at which the removed volume is simply rejected.<sup>9</sup> (23) Wash out the pipette well in the boiling water. (24) Add one volume of bacterial suspension to each of the serum dilutions beginning with the twelfth and passing up<sup>10</sup> to the first. (25) Mix at the time of each addition. (26) Wash out the pipette well in the boiling water. (27) Allow to cool somewhat, and take up a sample of the serum-suspension mixtures in due order beginning with that containing the greatest dilution of serum (12th depression) and continuing to that with the least. (28) Place each sample as taken up, in its appropriate tubule. (29) Examine each tubule with the hand lens and record as 'Immediate' the agglutination which has already taken place, with its degree, and the dilution of serum. (30) Place the tubules in the incubator for 2 hours at 37°C. (31) Take out the tubules and record for each dilution of serum the degree of agglutination which has occurred, in terms of *opacity* (a, b, c, d) of mixture. (32) Invert the tubules and fix the stand with tubules inverted, on the edge of a tin or wire basket. (33) Leave 30 minutes. (34) Record then for each dilution of serum the degree of agglutination, which has occurred, in terms of size and compaction of flocculi (stages 0-6). (35) Leave 24 hours at room temperature and repeat the same method of reading the result as used after removal from the incubator. (36) Calculate titre.

<sup>1</sup> All drawn from the same piece of glass tubing. <sup>2</sup> The tubules are made, in the process of drawing, with an extension which serves as a foot. In this way the bottom of the tubule is raised above the surface of the plasticine and is completely visible. <sup>3</sup> Any print will serve the purpose, as the comparison throughout of bacterial suspension is with a suspension of barium sulphate in 1 per cent sodium citrate, taken as the standard. <sup>4</sup> In the test as described a suspension of living organisms is used and this is standardized on each separate occasion. A formalinized suspension of dead organisms would have the advantage that, once standardized, it would serve for a considerable time without further standardization. <sup>5</sup> This is merely a rough means of obtaining a certain amount of uniformity in the suspension to be standardized and is not at all essential. <sup>6</sup> This mark would naturally be made much higher up than that indicating the 'unit-volume' previously referred to. <sup>7</sup> A certain amount of special manipulation is required to fill tubules of this bore with an unbroken column of fluid. It may be done by passing the pipette to the bottom of the tubule, filling the tubule by expulsion with the pipette in that position and sharp withdrawal of the pipette when emptied, or better when almost emptied. <sup>8</sup> In the process of taking up a unit volume of serum or serum dilution, and in mixing avoid carrying fluid above the mark, as that may leave there a quantum of serum which may be carried on to a much lower dilution and thus vitiate the results. <sup>9</sup> The dilutions of serum obtained are 1 in 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096. <sup>10</sup> In this way passage is from greater dilution to less and there is no need to wash out the pipette during the operation. The final dilutions of serum obtained in this series are 1 in 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096 and 8192.



## CONCLUSIONS.

1. The degree of agglutination for time of reaction or for dilution of serum can be, and should be, described in detail.

2. The procedure commonly used in agglutination is very variable. The factors involved should be fixed by agreement. The standardization of reagents is desirable.

## REFERENCES.

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# PRODUCTION OF HIGH TITRE SERA.

BY

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THE ordinary routine procedure connected with the manufacture of high titre sera can afford considerable information on such points as:—Differential methods of inoculation, dosage, antibody response, maintenance of titre, methods of standardization of reagents, mode of record of results and so on. All these points likewise have their bearing on the wider problems of immunization and immunity. The high titre sera which form the subject of the observations here recorded were almost wholly agglutinating sera and the antigens used were represented by a fairly wide range of pathogenic organisms. The points taken up might each of them form the subject of detailed research. They are also closely interrelated but it will simplify the treatment of the subject to consider them under separate headings as follows:—

## I. DOSAGE.

1. Limits of dosage.
2. Limitations of frequency.

## II. TITRE.

## III. DIFFERENTIAL METHODS.

1. Mode of introduction.
2. Single and successive doses.

## IV. FORM OF ANTIGEN.

1. Living or dead.
2. Dried or undried.

## V. ANTIBODY RESPONSE.

1. Suitability of animal, species, age, etc.
2. Specificity.
3. Effect of rest from dosage.
4. Non-specific immunization.

## VI. MAINTENANCE OF TITRE.

1. Without inoculation.
2. With inoculation.

## I. DOSAGE.

The dosage to be used will depend on the species of animal, its size, the mode of administration, the species of organism and so on.

I. *Limits*.—In goats it was found that a convenient series for intravenous inoculation was one of duplication of dose, with an initial dose equal to 1 mgm. of dried bacterial substance. Three doses of 1, 2 and 4 mgm. should be sufficient to give a good high titre serum. It was not found advisable to proceed much in advance of a dose of 4 mgm. The dosage was estimated with the help of the following opacity table :—

TABLE I.

*Brown and Kirwan\* (1915) showing the relation of opacity to weight and number of organisms in millions per c.c.*

Dilution of 1% barium sulphate in 1% sodium citrate.	Staphy- lococcus aureus, M. catarrhalis, Gono- coccus.	B. typho- sus.	B. coli.	B. para- typhosus A.	B. dysen- teriae (Flexner.	B. cholerae.
1-8 . . .	3374	2138	1951	2044	2633	9265
1-9 . . .	<b>3000</b>	1900	1734	1817	2341	8236
1-10 . . .	2700	1710	1560	1635	2107	7412
1-11 . . .	2454	<b>1555</b>	<b>1419</b>	<b>1487</b>	<b>1916</b>	6739
1-12 . . .	2250	1425	1300	1363	1739	6177
1-13 . . .	2075	1315	1200	1258	1621	5702
1-14 . . .	1928	1221	1115	1169	1442	5294
1-15 . . .	1800	1140	1040	1090	1405	4942
1-16 . . .	1687	1069	975	1022	1317	<b>4633</b>

The antique figures represent the numerical equivalent in millions, of 1 mgm. of dried bacterial substance.

\* Considerable variations in the equivalence of number of organisms and opacity occur, according to the method of enumeration used. But the correlation given in this table between opacity of suspension and weight of bacterial substance is not affected thereby.

TABLE II.

*Showing maximum lethal\* and maximum non-lethal† doses of living organisms by intravenous injection. The figures given indicate number of organisms in millions.*

Organism used.	LETHAL DOSE.		NON-LETHAL DOSE.	
	Goat	Fowl	Goat	Fowl
<i>B. dysenteriae</i> (Shiga)	30,640		15,320	7,660
<i>B. dysenteriae</i> (Flexner)	30,656		15,328	3,830
<i>B. typhosus</i>	15,780		13,150	11,400
<i>B. cholerae</i>	18,582		24,000	18,582
<i>Staphylococcus</i>	30,656		18,000	

\* Where such doses were fatal this was not due wholly to the toxicity or infectivity of the organism. The introduction of particulate matter capable of causing mechanical obstruction to capillaries is certainly also a factor in lethality.

† The table of maximum non-lethal doses serves to show what can be injected intravenously without causing difficulties due to mechanical effect, or to toxic or infective action. The maximum non-lethal dose is sometimes higher than the maximum lethal. This refers then to a different animal or to the same animal on a different occasion.

As regards size and repetition of doses there were distinct differences in toleration exhibited towards different organisms. The dysentery group of organisms has to be carefully administered in goats, while it would appear as if cholera and staphylococcus might be given intravenously with impunity. The injection given, whether an initial or subsequent dose, was always of living organisms except where it is specified to be a suspension of dried bacteria. With the laboratory strains used there seemed to be little danger to animals such as the goat and the fowl in using living organisms. A danger may be, however, that the animal is converted into a carrier. Much larger doses

still of dried bacterial substance were administered than of living bacteria.

TABLE III.

*Showing maximum lethal and maximum non-lethal doses of dried \* bacteria! growth administered in suspension and intravenously. The figures represent dried growth in milligrammes.*

Organism used.	LETHAL DOSE.			NON-LETHAL DOSE.		
	Goat.	Rabbit.	Fowl.	Goat.	Rabbit.	Fowl.
B. cholerae . . . .		(1) 20		32	10	10
		(2) 5				

\* The growth on an agar slope was simply taken off, with a platinum loop, placed in a watch glass and dried over sulphuric acid or sticks of caustic potash.

2. *Limitations of frequency.*—The frequency with which a goat or a fowl can be inoculated intravenously seems to be indefinite. That is to say there seems to be no danger from the mere repetition of intravenous inoculation, aside from the effect of the organism used. Goats received as many as 20 to 30 intravenous inoculations and fowls as many as 12 without apparent deleterious effect. The spacing between injections was 7 or 10 days. Special trials were made of rapid production of anti-serum and also of the introduction of a pause in the series of inoculations. The 7 or 10 day interval between doses was used without any reference to the usual recommendation of repetition of dosage, which is dependent on restoration of weight of animal, etc. This procedure in which a definite type of serial dosage was employed and time intervals were strictly adhered to, has the merit of a uniformity which is very useful for purposes of comparison.

## II. TITRE.

In another paper in this number of the Journal I have discussed questions of definition and mode of record of titre. These I need not go over again except to give a short description of the mode of record of titre and the conditions under which it is recorded.

The suspension used is standardized. The serum suspension mixture is placed in the incubator at 37°C. for 2 hours and thereafter left at

room temperature for 24 hours before the result is read. Two characters enter into the reading :—(1) Opacity of the mixture, which is read before inverting the test tubules. The opacity readings are given by letters *a, b, c, d*. The letters *c* and *d* represent different grades of complete agglutination; *b* represents what is equivalent to macroscopically evident, but partial, agglutination, and *a* represents a degree of opacity which is the same as the salt control. (2) Stages of agglutination present. The tubules are first inverted and left for 30 minutes to allow the deposit to distribute itself by simple gravitation. At the end of the time the tubules are restored to the original position and a reading made with a hand lens magnifying 10 diameters. The stages recorded are 6, 5, 4, 3, 2, 1, 0, respectively.

Stage 6 = stage of agglomerated mass, which still coheres as a single mass even after inversion.

Stage 5 signifies the presence of large masses of agglutinated organisms.

Stage 4 signifies the presence of small masses.

Stage 3     "     "     "     "     coarse particles.

Stage 2     "     "     "     "     fine particles.

Stage 1     "     "     "     "     a ground glass or grained appearance which indicates incipient separation of agglutinated organisms into particles.

Stage 0 signifies that no apparent agglutination has taken place. These stages are usually present more than one at a time. Thus a titre,  $512 = 654/b$ , signifies that for the dilution of serum 1-512 stages 6, 5 and 4 were present but not 3, 2, 1 or 0, and that the opacity showed definite macroscopic reduction from that of the control tubules. I have found it convenient as a method of description, and economical of space, not to give the whole of the findings for all the dilutions (1-4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192) but simply to give as the titre (1) the highest dilution which gives complete or nearly complete agglutination and (2) the highest dilution which gives macroscopically evident, but partial, agglutination. In each of these cases (1) and (2) the stages of agglutination and opacity are given. Thus a titre expressed as  $\begin{matrix} (1) 256 = 65/c \\ (2) 4096 = 432/b \end{matrix}$  signifies that up to dilution 1-256 but not beyond the agglutination was complete or nearly complete, and that it was macroscopically evident although partial up to 1-4096 but not beyond. The stages are run together as if they were whole numbers, 65 signifies stages 6 and 5.

## III. DIFFERENTIAL METHODS.

1. *Mode of introduction of antigen.*—There are only three modes in common use—the intravenous, the intraperitoneal, and the subcutaneous. These represent usually a descending scale of efficiency in regard to rapidity of production of anti-serum. The subcutaneous is so unsatisfactory that it may be abandoned altogether except for the case of highly pathogenic organisms where it undoubtedly has the advantage of greater safety.

TABLE IV.

*Showing the comparative effect of various methods of introduction of antigen. The interval between successive inoculations is 10 days, and the total number of doses is four. Each successive dose is double that of the previous one. The titre shown is the highest dilution of serum which gives naked eye agglutination after 2 hours at 37°C., followed by 24 hours at room temperature.*

Mode of introduction of antigen.	GOAT.			RABBIT.			FOWL.		
	Antigen.	Initial dose.	Titre.	Antigen	Initial dose.	Titre.	Antigen.	Initial dose.	Titre.
Intravenous . . .	Dried cholera.	4 mgm.	4096*	Dried cholera.	5 mgm.	4096*	Dried cholera.	0.25 mgm.	8192*
Intraperitoneal . . .	..	..	..	Dried cholera.	10 mgm.	4096*	Dried cholera.	0.5 mgm.	8192*
Subcutaneous . . .	Living typhoid	1,555,000,000	64	Dried cholera.	20 mgm.	1024	Dried cholera.	1 mgm.	128

\* Limit not determined.

2. *Size and repetition of dose.*—The experience gained so far in quite a small number of trials seems to show that the method of inoculation by an intravenous dose on three successive days, with rest for a week, and then repetition of the former dosage resulted, in fowls, in the rapid production of a high titre serum. The attainment of high titre is not, however, so very much more rapid than the method of intravenous inoculation with 7 or 10 day intervals. Again the use of a single large dose intravenously or of a fixed large dose repeated at 10-day intervals afforded no evidence of improvement over the usual method. A method which



has been found useful and efficacious by Iyengar (1919) for anti-sheep hæmolytic sera in rabbits is as follows :— The method consists in giving intravenous injections of 2 c.c. of increasing strengths from a 5 up to 30 per cent suspension of centrifuged deposit of washed erythrocytes at intervals of 72 hours—5 per cent increase of strength being given on each successive occasion.' The titres (complete hæmolysis) obtained in 10 rabbits varied from 1-3000 to 1-7000.

The continuation of inoculations over a long period is not to be recommended, at all events in goats immunized for the production of agglutinating sera. The titre is not increased thereby. It shows, on the contrary, some tendency to decrease in potency. But perhaps the chief disadvantage of continuance is that the serum would seem to become less specific, and to agglutinate allied organisms in greater degree. It is possible that this may represent a continuance of immunization to co-agglutinogens, after the titre to the specific agglutininogen has reached its fixed limit. The response then to continued immunization would, on this hypothesis, take the form of maintenance (or some loss) of specific agglutinin and increase in production of non-specific agglutinin. The following table gives the results which I have obtained by the use of different methods in fowls

TABLE V.

*Showing the results obtained in fowls from the use of different sized doses of dried cholera antigen intravenously and from the employment of different intervals of administration. The titre shown is that given ten days after the inoculation to which it refers. The times given in the final column of the table include the ten days allowed, after inoculation, before the titre is taken.*

No.	Type of administration.	No. of dose.	Interval between successive doses. Days.	TITRE.			
				Dilution.	Result.	Dose mgm.	Time to completion. Days.
1	Small dose doubled at each successive inoculation.	1st	..	512	432/c	0.25	..
				2048	320/b		
		2nd	10	..	..	0.5	..
		3rd	10	..	..	1.0	..
		4th	10	8192 *	65 c	2.0	40
2	Rapid immunization on three successive days with an interval of a week and then repetition for another three successive days.	1st	..	..	..	0.25	..
		2nd	1	..	..	0.5	..
				1024	54 c	1.0	12
		3rd	1	2048	5432/b		
		4th	8	..	..	2.0	..
				..	..	4.0	..
		5th	1	4096	54 c	8.0	30
		6th	..	8192	4320/b		
3	Single large dose	1st	..	128	54 c	10	10
4	Fixed large dose repeated	1st	..	256	432/c	10	..
				512	4320/b		
	....	2nd	10	512	5432 c	10	..
				1024	54320/b		
	....	3rd	10	256	5432/c	10	..
				512	4320/b		
	....	4th	10	32	5432 c	10	40
				256	5432/b		

The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination was obtained after 2 hours at 37°C. and 24 hours at room temperature. In no case did the serum of the animal before inoculation give a macroscopically visible agglutination above a dilution of 1.16. The method of recording values is that described in my paper in this number of the Journal, on Technique of Agglutination.

\* Dilution not carried to limit.

#### IV. FORM OF ANTIGEN.

1. *Living or dead organisms.*—No very detailed quantitative and comparative trials were made to determine whether a suspension of

living organisms showed superiority as antigen over a suspension of dead organisms. It was evident, however, that a suspension of dead organisms could produce a satisfactory agglutinating anti-serum. It is certainly convenient to keep a prepared suspension for inoculation instead of making it up afresh on each occasion. The question whether there is a distinct advantage in using the living organisms must be left over for further test. The results obtained with a dried cholera antigen in fowls were extremely satisfactory. What was determined, however, was that the living laboratory strains available could be administered intravenously in goats and fowls both as initial and subsequent doses without any danger. These organisms were:—*B. typhosus*, *B. paratyphosus* A and B, *B. dysenteriae* (Shiga), *B. dysenteriae* (Flexner), *B. dysenteriae* (Y Hiss), *B. enteritidis* (Gaertner), *B. cholerae*, *B. coli* and *staphylococcus*. Living *Micrococcus melitensis* was not injected into goats owing to the susceptibility of these animals to infection.

TABLE VI.

*Showing typical results obtained by intravenous inoculation of certain dead and living organisms in goats.*

Organisms.	Initial dose in millions.	RESULTING TITRE.*		REMARKS.
		Dead organisms.	Living organisms.	
<i>B. dysenteriae</i> (Flexner).	479	1024 = 653/c 2048 = 652/b	..	The resulting titre in this case is given for a single inoculation only. An easily agglutinable strain. After a single inoculation only. An easily agglutinable organism.
	1915	..	8192 = 65/c †	
<i>B. typhosus</i>	1555	..	2024 = 65/c 8192 = 652/b	Three inoculations at 10-day intervals: each dose double the preceding one.
<i>M. melitensis</i>	700	1024 = 65/c 4096 = 42/b		Seven inoculations at 10 day intervals, each dose double the preceding one.

\* The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination was obtained after 2 hours at 37 C. and 24 hours at room temperature. In no case did the serum of the animal before inoculation give a macroscopically visible agglutination above a dilution of 1-16.

† Dilution not carried to limit.

2. *Dried organisms*.—The examples taken were *B. typhosus*, *B. dysenteriae* (Shiga), and *B. cholerae*. Most of the organisms used for the production of high titre sera are killed in the process of drying and the process of immunization with this antigen is therefore representative of use of killed organisms. The advantages obtained by the use of this antigen are :—(1) It does not involve the use of any antiseptic as a preservative. (2) It does not entail any interference with chemical constitution other than simple desiccation at room temperature. (3) It forms a very homogeneous suspension when ground up in 0·85 per cent salt solution. (4) It is tolerated in large doses by intravenous injection. (5) It preserves its characters as antigen for a very long period. These represent very considerable advantages over the suspension of those organisms which have been killed by heat and to which carbolic acid or other antiseptic is added as a preservative.

*Mode of preparation and use of dried antigen.*

(1) Weigh an empty sterile watch glass or small test tube. (2) Remove carefully with platinum loop the growth<sup>1</sup> from an agar slope. (3) Weigh the watch glass with its contents.<sup>2</sup> (4) Place the watch glass in a desiccator<sup>3</sup> over sulphuric acid, or caustic soda sticks. (5) Weigh the watch glass when the antigen is completely dried<sup>4</sup>, and determine by calculation the weight of dried antigen obtained. (6) Remove a quantity of dried antigen and place in a sterile agate mortar. (7) Re-weigh the watch glass and determine by calculation what quantity of dry antigen has been removed. (8) Fill a sterile 1 c.c. syringe with 0·85 per cent sterile salt solution. (9) Add 5 drops from the filled syringe to the dry antigen in the mortar. (10) Leave the whole to soak overnight in moist chamber. (11) Fill a sterile 1 c.c. syringe with 0·85 per cent sterile salt solution, and reject the number of drops already used for soakage purposes. (12) Grind<sup>5</sup> up the antigen to make a suspension with the remainder of the fluid contained in the syringe.

<sup>1</sup> It is a very distinct advantage, as regards facility of removal, to, have agar slopes dry before sowing. This can be effected by leaving them in an incubator overnight in the inverted position. The wool will soak up the water of condensation.

<sup>2</sup> This is not really necessary as it gives the weight of organisms in the moist condition, whereas all that is required for standardization is the weight when dried.

<sup>3</sup> The desiccator may be vacuumized, if particularly rapid desiccation is required.

<sup>4</sup> As given by constancy of weight in two successive weighings, sufficiently separate in point of time.

<sup>5</sup> This is very easily done when the method of preliminary soakage is adopted.

(13) Fill the syringe with the suspension obtained. (14) Inject intravenously the dose required.

TABLE VII.

*Showing result of use, and duration of efficacy as antigen of dried bacteria, by intravenous inoculation.*

Dried organism.	Duration of preservation.	Animal.	Initial dose in mgms.	Resulting titre.*	REMARKS.
<i>B. typhosus</i>	3 years	Fowl.	10.1	1096† = 65/d	Four doses, given at irregular intervals. Maximum dose = 31.7 mgm.
<i>B. dysenteriae</i> (Shiga).	3 years	Fowl.	9	....	The result was initiated, owing to the organism showing a certain amount of spontaneous agglutination. Five doses were given in all at irregular intervals. Maximum dose = 14 mgm.
<i>B. cholerae</i>	At least one month.	Rabbit	5	1024 = 43/c 4096 = 40/c	Result after two doses of 5 and 10 mgm. The animal died after the third dose of 20 mgm.
<i>B. cholerae</i>	At least one month.	Fowl.	0.25	8192† = 65/c	Three doses with two-fold increase of each succeeding dose. Interval = 10 days.
<i>B. cholerae</i>	At least one month.	Goat.	1	1048 = 43/c 4096 = 432/b	Three doses with two-fold increase of each succeeding dose. Interval = 10 days.

\* The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination were obtained after 2 hours at 37°C. and 24 hours at room temperature. In no case did the serum of the animal before inoculation give macroscopically visible agglutination above a dilution of 1:16.

† Dilution not carried to limit.

TABLE VIII.

*Showing comparison of use as antigen of equivalent quantities of dried and undried (living) cholera antigen by intravenous injection in fowls. Intervals between injections = 10 days. Number of doses = 4, with two-fold increase on each successive injection.*

	Dose.	Resulting titre.*	REMARKS.
Dried . . . .	0.25 mgm.	8192† - 65/c	The dose here given was very small for dried antigen. ....
Undried . . . .	1,158,000,000	8192† = 432/c	

\* The dilutions given are the highest in which complete agglutination was obtained after 2 hours at 37°C. and 24 hours at room temperature.

† Dilution not carried to limit.

## V. ANTIBODY RESPONSE.

1. *Suitability of animal.*—That it is not immaterial what animal is used for the production of a high titre serum is—even if not otherwise a known fact—suggested by the following attempt to attain an anti-sheep hæmolyzing erythrocyte serum from fowls.

TABLE IX.

*Showing the fruitlessness of the attempt to obtain a hæmolytic serum in the fowl by intravenous injection of washed sheep erythrocytes.*

Serial No.	Quantity of erythrocytes injected, c.c.	Dilutions of centrifuged deposit used.	Interval between successive injections.	Number of injections.	RESULT.
1	0.1 : 0.2 : 0.4 & 0.8	Undiluted	7	4	Serum with some agglutinating power but feeble hæmolytic action.
2	2	5,10,15,20 & 25 per cent	3	5	ditto.
3	1	ditto	ditto	ditto	ditto.
4	0.5	ditto	ditto	ditto	ditto.
5	0.2	ditto	ditto	ditto	ditto.

The species of animal used for the production of high titre agglutinating sera were goats, rabbits and fowls. The advantages and disadvantages of using one or other of these animals may be set down, in so far as the trials made have demonstrated them. It may be well to say that a great many more trials would have to be made to enable one to dogmatize on the subject, or to give greater precision to the statements.

#### *I. Goat.*

- (1) The jugular vein is large and easily entered.
- (2) The yield of serum is large : four or five hundred cubic centimetres of blood may be withdrawn at one time, while the yield of serum varies from 50 to 60 per cent.
- (3) The animal continues to yield high titre serum for a long period.
- (4) The titre of the serum obtained is not so high as that of rabbits, but is sufficiently so for all practical purposes, especially as it is used undiluted.
- (5) The serum obtained may not be so highly specific as that of rabbits.
- (6) The animal is easily procurable in India, but costs more than rabbits or fowls to keep.

#### *II. Rabbit.*

- (1) The ear vein in small rabbits is not large and requires some skill to enter.
- (2) The yield of serum is not large. Fifty to sixty cubic centimetres of blood is all that can be expected in the way of yield.  
The percentage yield of serum is no greater than in goats.
- (3) The animal is usually killed when the serum shows the necessary titre.
- (4) The titre obtainable being high, the serum is usually diluted for use. The titre is in this way lowered—probably distinctly below the titre of the undiluted goat serum. Even with dilution the amount obtainable will not compare with that from goats.
- (5) The serum obtained may be more highly specific than that of goats.
- (6) The animal is not easily procurable in India, and is expensive.

#### *III. Fowl and guinea-fowl.*

- (1) The wing vein of the fowl is small but is distinctly larger than that of ordinary sized rabbits.



(2) The yield of serum is not large. Thirty-five to forty cubic centimetres of blood is all that it has been found possible to withdraw by syringe. The percentage yield of serum is less than in either goats or rabbits.

(3) The animal may be killed when the serum shows the necessary titre but amounts of blood up to 10 c.c. can be easily obtained sterilely as required from the wing vein.

(4) The titre obtainable is high.

(5) The specificity of the serum is satisfactory.

(6) The animal is easily procurable in all parts of the world and is cheap both to buy and to keep.

TABLE X.

*Showing yield of blood and serum obtained from goats in a series of trials.*

No.	Blood c.c.	Serum c.c.
1-1	431	182
4-9	410	240
5-13	397	200
5-13	520	210
6-17	410	260
9-25	225	106
7-33	420	220
11-49	400	263
14-57	450	230
21-101	212	170

2. *Specificity of serum.*—The statement has been made above that the serum of goats may not be so specific as that of rabbits. This, however, requires qualification. It may apply to goats which have been under immunization for a long time. The titre of serum from goats which have only received in all three or four inoculations is just as specific as that of rabbits. Unfortunately the titre attained with this number of inoculations does not come up to that of the rabbit for the same number of inoculations. This may, however, in my findings, be dependent on the doses relative to size of animal, which were used.

## Production of High Titre Sera.

TABLE XI.

Showing specificity of serum (1) from goats and rabbits (2) from goats inoculated with a few doses only as compared with those inoculated with many doses.

Animal.	Organism.	Number of inoculations.	TITRE* OF SERUM.					REMARKS.
			Typhoid.	Para A.	Para B.	Shiga.	Flexner.	
Rabbit .	Typhoid	..	512=654/c 1024=4320/b	4=0/a	4=650/b	4=0/a	32=652/c 64=10/b	1-10 with carbolyzed serum diluted salt solution.
	Para A	..	32=65/c 256=4320/b	32=65/c 4096=650/b	8=65/c 1024=40/b	4=0/a	4=65/c 8=50/b	
	Para B	..	128=65/c 512=5420/b	8=6/c 64=50/b	128=65/c 2048=640/b	4=0/a	8=6/c 16=30/b	
	Shiga	..	4=40/b	44=0/a	4=0/a	1024=62/c 8192=62/b	4=6/c 32=40/b	
	Flexner	..	4=620/c 16=540/b	4=510/b	4=0/a	4=0/a	128=6532/c 256=5420/b	
Goat .	Typhoid	4	1024=65/c 8192=652/b	16=62/c 64=620/b	16=65/c 64=520/b	16=520/b	512=52/c 1024=50/b	
	Flexner	4	32=20/b	16=520/b	16=520/b	256=52/c 512=520/b	8192=65/c	
	Shiga	4	32=20/b	16=20/b	16=20/b	1024=65/c 4096=50/b	16=65/c 64=30/b	
Goat .	Typhoid	13	512=65/c 2048=6542/b	8=65/c 128=30/b	8=654/c 128=40/b	16=65/c 256=40/b	4=65/c 16=130/b	
	Para A	21	1024=65/c 4096=5432/b	1024=65/c 4096=40/b	128=5432/c 1024=40/b	8=65/c 64=420/b	8=65/c 16=53/b	
	Para B	20	512=65/c 2048=63/b	512=54/c 1024=432/b	1024=65/c 4096=650/b	4=65/c 8=540/b	4=652/c 8=40/b	
	Flexner	22	4=5/c 32=40/b	4=65/c 64=60/b	4=65/d 16=543/b	8=65/c 16=50/b	1024=65/c 4096=5420/b	

\* The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination were obtained after 2 hours at 37°C. and 24 hours at room temperature.

3. *Effect of rest.*—On the supposition that the cells producing antibodies may become overtaxed by successive inoculations of antigen it would seem reasonable to suppose that an intermission of inoculation would be beneficial and that their resumption after rest would be very quickly followed by restoration of titre, if it had fallen, or even a further elevation.

TABLE XII.

*Showing effect of rest from intravenous inoculations in goats, and the effect of resumption. Two doses were given on resumption, at an interval of 10 days. Titre taken 10 days after each dose.*

Organism.	Duration of rest.	Dosage after rest.	TITRE.*		
			Before commencement of rest.	At the end of rest.	After resumption of dosage.
Y-Hiss . .	3 months	(1) 4000 M	512=5/c	256=65/c	1024=65/c 4096=54320/b
		(2) 24000M	1024=6540/b	1024=420/b	512=65/c 2048=430/b
Cholera . .	2 months	(1) 4 mgm. dried.	1024=65/c	512=654/c	1024=5/c 8192=5432/b
		(2) 4 mgm. dried.	8192=54/b	2048=40/b	512=5/c 8192=5432/b

\* The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination was obtained after 2 hours at 37°C. and 24 hours at room temperature. Rest, it would seem, results in slow loss of titre, but the original titre, or a higher one, can be obtained by a single injection after such periods as 2 or 3 months without injection.

4. *Non-specific immunization.*—The tendency among bacteriologists at present is towards the multiplication of types within the species—‘serological types’ and subtypes. It is well to keep in mind, however, that a common chemistry may apply very largely to lowly organisms like bacteria and that a considerable amount of non-specific immunization may be involved in the production of immune sera. The endeavour to get a clear cut specificity may not be wholly sound. We may conceive a given group of organisms to represent continuous variation within a particular universe. Whether the universe is to be regarded as genus or species we may leave aside. The point to be made is as to

*continuity* of variation. It is possible, indeed probable, that even although the variation is continuous through the group and connects up the several numbers with one another it may not for special conditions, such as disease, show an even frequency of occurrence of the variates. The manufacture of the 'serological type' seems to be partly admission of variation within the species and partly the ordinary human endeavour to subdivide minutely. The question, however, is an important one when it results in insistence upon separation of antigenic or immunizing effect with separation of type. Here again the subject is a large one in itself and I do not propose to take it up in the way which its importance would demand.

TABLE XIII.

*Showing the effect of immunization of already immunized goats with a single intravenous dose of a second organism of different species.*

First organism.	Second organism.	Interval between the termination of the first immunization and commencement of the second.	Dose.	TITRE* TO SECOND ORGANISM.	
				Before dosage.	After single dose.
Staphylococcus aureus.	B. dysenteriae (Flexner).	3 months	479M	16=65/d 32=642/b	2048=62/c 4096=6420/b
B. coli . . .	B. dysenteriae (Flexner).	2 months	479M	16=64 c 32=30/b	2048=632 c 4096=520 b

\* The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination was obtained after 2 hours at 37°C. and 24 hours at room temperature.

#### VI. MAINTENANCE OF TITRE.

There comes a stage at which further inoculation of antigen appears to give rise to little or no appreciable increase of the titre. This is the stage of 'fixed titre.' In many cases, however, a slow increase does go on. In other cases, however, it would seem as if decrease began to take place. Maintenance of titre is accomplished in goats in various ways, as for example by (a) subcutaneous injection after the titre has been raised by intravenous inoculation; (b) inoculation of a fixed medium dose subcutaneously at definite intervals; (c) inoculation of a

fixed medium sized dose intravenously at definite intervals: (d) inoculation of very small doses intravenously.

TABLE XIV.

*Showing trials of methods of maintenance of titre of serum in the goat.*

Organism	Method.	Number of inoculations for maintenance.	TITRE.*		REMARKS.
			Original.	Final.	
Paratyphoid B.	No further inoculation	None.	4096†=5 c	256=65 c 8192=430 b	Control.
Typhoid	Subcutaneous ] inoculation with two-fold increase of dose every 10 days, starting with 4000 million.	7	1024=5/c 2048=543 b	4096=65432/c 8192=65432/b	Titre not only maintained, but increased.
Paratyphoid A.	Intravenous inoculation with a fixed dose of 4000 million every 10 days.	13	512=5/c 1024=540/b	1024=65/c 4096=40/b	Titre not only maintained, but increased.
Paratyphoid B.	Intravenous inoculation with a fixed dose of 40 million every 10 days.	13	4096†=65/c	2048=5/c 8192=430/b	Slight decrease of titre.

\* The dilutions given are the highest in which (1) complete agglutination and (2) macroscopically visible agglutination was obtained after 2 hours at 37°C. and 24 hours at room temperature.

† Dilution not carried to limit.

The inference from these trials would seem to be that once a satisfactory titre is obtained, maintenance can be effected by simple subcutaneous injection. Whether it is desirable to do so indefinitely is another question.

#### CONCLUSIONS.

1. A convenient series of doses for the intravenous inoculation of goats with living organisms is that number of organisms corresponding to 1, 2, 3, 4 mgms. of dried bacterial substance. Fowls can stand large doses intravenously.

2. The best results from treatment consisted of continuous insect control by ground spray.

3. A 7 to 10-day interval is a convenient one in which frequent insecticide and seed be applied to grow and build without extensive reworking of beds or soil.

4. Continuous application of nitrogen as a soil-poll-growth was more efficient for the production of seed than fertilization. Subsequent phosphorus and potassium inputs have the same effect on seed of limited benefit.

5. The long time of being available to the crop, the importance of shading and the growth of weeds in the ground. The growth of the ground crop has to be more carefully observed than others.

6. Suppression of weed organisms obtained from laboratory studies may be given all other treatments in plots.

7. Good growing and growing to seedling need to overcome inhibition of seedling of killed organisms.

8. Vegetation, simply, increased, was a very satisfactory, very stable, and very efficient system.

9. Reduced yield of seed of high seedling by the growing of the ground. There are many other systems available for the production of high seed yield, but the best system is to grow the seedling. There are no other systems available for the production of high seed yield. There are no other systems available for the production of high seed yield.

10. The best system for growing seedling is to grow the seedling. There are no other systems available for the production of high seed yield.

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## PELLICLE FORMATION IN BROTH CULTURE BY *BACILLUS CHOLERÆ*

101

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The outbreak of war necessitated the production of bacteriological materials in other countries which had formerly been produced almost entirely in Germany. It was natural then that variations in the composition of chemical products should occur. These again bacteriological investigations have to be undertaken in localities in which they had not been to any great extent carried out before and also under conditions of considerable difficulty. It is not surprising therefore to find that when the common reactions described to identify bacteriological species failed to appear, there was a tendency to ascribe the results to the unreliability of the materials used or to the occurrence of a rapid and new strain of organisms. The case of the student under is illustrative of this tendency. While I do not propose to question the correctness of the line on which he proceeded as to the occurrence of new strains or impurities in the culture medium used he based, I have found but explanation at least of the conditions required for the production of the characteristic spiculate pellicle formation by the chosen strain. The medium reacting for the ordinary laboratory medium + 10 Kilo Litmus. This reaction is a typical sugar broth, where the substrate is glucose, is unsatisfactory for pellicle formation by *B. cholerae*. Many test-tubes refer to alkalinity as a necessary for pellicle formation but do not state the any further detail. I have used a series of media to place the reactions (Eyre's study) varied from -11.5 to +11. These were some



## 702 *Pellicle Formation in Broth Culture by B. cholerae.*

from 24-hour agar cultures and themselves incubated aerobically and anaerobically for 24 hours. The result was striking and is shown in plate LXIV. It served to demonstrate that such a very important specific character as pellicle formation was intimately bound up with the reaction of the medium. This is clearly shown in the following table:—

TABLE

*Showing degree of pellicle formation and growth by B. cholerae in nutrient tryptic broth of varying degree of reaction (Eyre's scale).*

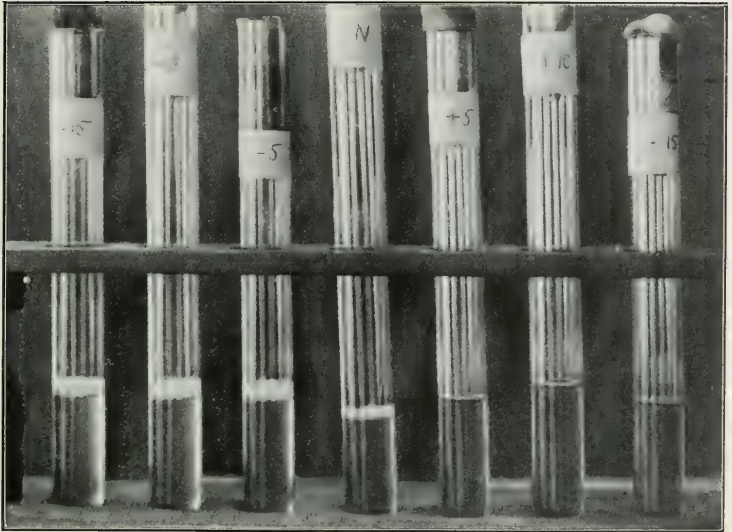
Reaction of broth.	—15	—10	—5	0	+5	+10	+15
Degree of pellicle formation ..	Marked	Marked	Marked	Slight	Nil	Nil	Nil
Degree of growth ..	Good	Good	Good	Fair	Weak	Weak	Very weak.

That degree of alkalinity alone may not be the whole explanation, however, is suggested by the following experiment. In this the Douglas tryptic digest broth was replaced by a Lemco Witte peptone broth with the same degrees of reaction. The conditions of sowing and incubation were the same as before, but in no case did any pellicle formation occur. The actual growth of the organism likewise was very weak in the alkaline broths and practically *nil* in the acid. This seems to suggest the necessity for a highly nutritive medium in order that pellicle formation may show. As the same appearances were given in both aerobic and anaerobic cultures, it would seem as if pellicle formation was not so much a phenomenon of surface growth by an organism greedy of oxygen as one of degree of nutrition afforded by the medium used. The experiments were not confined to the use of one strain only. Five strains in all were used, two Mesopotamian and three Indian, and all gave the same results. Another specific characteristic of the growth of *B. cholerae* is the formation of indol. This was not fully investigated but it was found that the indol reaction of this organism was only given by the growth in alkaline broths and not at all in the acid.

### CONCLUSIONS.

1. Many of the growth characters on which the differentiation of species is made depend on conditions which are by no means fully worked out.

PLATE LXIV.



24-hour agar culture of *B. cholerae*, showing pellicle formation in alkaline broth and none in acid broth.

K. R. K. IYENGAR—Pellicle Formation in Broth Culture by *Bacillus cholerae*.



2. The failure to obtain specific reactions in organisms under test does not always justify the conclusion as to the divergence of the organism from the type or as to the imperfection of the nutrient media.

3. Pellicle formation by the cholera vibrio and probably indol formation is dependent on the alkalinity of the medium used.

4. The highly nutritive character of the medium is probably a determinary factor in the production of pellicle by *B. cholerae*.

# THE PREPARATION OF A SIMPLIFIED CULTURE MEDIUM FOR FIELD WORKERS.

BY

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ONE of the principle difficulties met with by bacteriological field workers, who from the nature of their employment are necessarily usually away from their headquarters, is the question of culture media. This question while possibly only of secondary importance in the West is certainly a primary consideration in the East where it is easily possible for a man to be many days' journey away from supplies.

In cases where a man is dependent for transport upon mules the supplies of culture media with which he sets forth are necessarily limited by the amount of space he can spare in panniers for that purpose. He is faced therefore by the prospect, sooner or later, of making his own culture media or of depending on subsequent supplies from headquarters following after him.

The usual methods of preparation of nutrient broth or nutrient agar are well known to every one. They are briefly recapitulated below for the sake of comparison later with the simplified preparation.

Five hundred grammes of lean meat are minced and boiled with 1 litre of water for two hours. It is then strained through a clean linen cloth and the filtrate immediately filtered through filter paper. The volume is again made up to 1 litre and 10 grammes of peptone and 5 grammes common salt are added. The whole is then again boiled and afterwards neutralised with a 4% solution of sodium hydrate and the

neutralisation continued until just alkaline to litmus. The fluid is then filtered again and made up to the original volume.

A much more convenient process is to use Liebig's extract of meat instead of the meat extract described above—2 grammes to the litre is the usual strength employed. Peptone is added as before.

For solid media agar is added to the above usually in the proportion of 20 grammes to the litre, though if roll cultures are desired this amount may be increased to 40 grammes per litre.

The next method is due to the work of Douglas (*Lancet*, Vol. II, 1914, p. 891) and is probably the one most generally in use at the present time as with slight variations in reaction, etc., it gives a luxuriant growth of most organisms.

Briefly it is as follows :—Five hundred grammes minced lean meat, 1 litre water. Boil. Cool to 45°C. Add 20 c.c. pancreatic extract (for preparation see Cunningham, *Indian Journal of Medical Research*, Vol. VI, 1918, p. 147). Digest for 4 hours at 37°C. Filter through muslin to remove any fat. Add 0.5 c.c. glacial acetic acid. Heat to 110°C. or boil for 15 minutes. Filter to remove mince. Make slightly alkali to litmus. Add 1.25 c.c. of 10% calcium chloride solution. If solid medium is required add 2 to 3% agar as before.

The above preparation is considerably cheaper than the first one given, dispensing as it does with the use of peptone. Both methods, however, are distinctly tedious and simple as they may sound they require a certain proficiency of technique to carry them through to a satisfactory conclusion.

In the course of previous work it was found possible with Douglas' method to replace meat by caseinogen at once as a step towards cheapness, saving of labour and the solving of storage difficulties. This work has now been continued with a view to the further simplification of the preparation of culture media and the results are collected in the present communication. Caseinogen culture medium when carefully prepared with a reaction of +10 yields an exceedingly luxuriant growth of *B. typhosus* and *B. cholerae*.

According to Malone, if the reaction be adjusted to +20 to +40 and pigeon's blood added in the way he describes a good growth of Pfeiffer's bacillus, which is a notably difficult organism, may be obtained.

It is a well known fact that caseinogen contains large quantities of amino-acids which are now considered as essential for the growth of

bacteria. Hence the first step towards a simplified medium for field work seemed to be the preparation of these substances in a dry state which was rendered feasible by the work of Dakin.

The main question to be solved was whether amino-acids so prepared would be sufficient in themselves to yield a nutritive medium or whether they would require the addition of peptone, a question of importance as regards expense.

*The preparation was as follows:—*A substrate containing 10% of caseinogen in a 0·8% aqueous solution of washing soda was digested with 0·5% pancreatic extract (for preparation see Cole, *Lancet*, July 1, 1916) at 37°C. for 24 hours.

It was afterwards filtered through muslin and neutralised, a portion of the filtrate was tested for nutritive efficiency and the remainder concentrated on a water bath. This was then transferred to an extraction apparatus and extracted with butyl alcohol according to the instructions given by Dakin. (*Jour Biochem.*, December, 1918, p. 290). The substance thus prepared was filtered off and dried in a desiccator, powdered and was then tested for nutritive value as described below. The following concentrations were made up using tap water or normal saline as diluent:—

1, 2 5, and 10%. To each of these was added 4% desiccated agar (for preparation see Cunningham, *Indian Journal of Medical Research*, April, 1919).

No peptone or extractives was added. Prepared in this way excellent results were obtained with *B. typhosus* but the method was somewhat laborious, the extraction alone taking a considerable amount of time. For production on a large scale therefore some simplified process of manufacture was required. It was decided to concentrate the products of digestion as far as possible on the water bath, a paste of the consistency and colour of Liebig's extract of meat being thus obtained, and on testing this for nutritive value in the manner and concentrations described above, it was found to be quite as efficient as the more highly purified product obtained by Dakin's method. It will keep in the form of this paste for a considerable period of time (at least six months which is the longest period so far under observation) without deteriorating in nutritive value or becoming contaminated. Hence the valuable ingredients would appear to be comparatively stable in character and not easily destroyed by oxidation.



It is essential that the product should be kept in stoppered jars as it is hygroscopic, although if left exposed until no further moisture is taken up it appears to retain its nutritive powers unimpaired.

It may be further dried by storage for some time in a desiccator over sulphuric acid when a brittle resinous mass is obtained which may be powdered, yielding a hygroscopic yellow powder. For practical purposes it would seem to be unnecessary to proceed to this stage.

It is not necessary to digest the caseinogen for long periods of time and in later preparations the concentrated filtrate from a four hours digest was found to be as effective as one from a five or ten days hydrolysis.

This is better illustrated by the following table which also demonstrates the excellent growth of *B. typhosus* which may be obtained. As stated before the powder was made up either with tap water or saline in 1, 2, 5 and 10% strengths and 4% desiccated agar added, no addition of peptone or extractives was made.

TABLE

*Showing yield of B. typhosus on agar medium containing as basis the dry concentrated products of a tryptic digestion of caseinogen continued for—*

4 hours.  
24 hours.  
48 hours.  
5 days.  
10 days.

Measured in 100 million per square c.m.

Concentration.	4 hours.	24 hours.	48 hours.	5 days.	10 days.
1 %	..	13.36 13.36 13.36 14.20	14.50 17.81 14.50 14.50	13.36 16.44	15.26 16.44
2 %	..	26.75 32.87	26.72 26.2 26.72 26.72	26.75 26.75	26.75 28.50
5 %	..	47.50 64.12 64.12 50.00	64.15 58.41 47.50	64.12 64.12	58.31 58.31
10 %	.	70.00 70.00	70.00 70.00	70.00 70.00	70.00 70.00

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The controls were 1. Broth before concentration which showed that no deterioration in bacterial growth was apparent if a 2% strength of dry substance was used.

2. Dry powder plus peptone which demonstrated that no advantage was to be gained by the addition.

3. Dry powder plus extractives also showing no advantage.

From the above table it is obvious that excellent growths of *B. typhosus* may be obtained on medium consisting of—

1. Powder from the concentration of a tryptic digest of a 10% caseinogen substrate.

2. Desiccated agar.

3. Tap water or saline.

For 1 it is suggested that the name 'trypsinoids' be used for the sake of convenience.

It is apparent that such culture medium materials at once solve the difficulties of transport and the necessity for large stores of supplies as both 1 and 2 are exceedingly compact. They are all exceedingly cheap and if a field worker be possessed of a small autoclave the whole preparation of media may be carried out in an exceedingly short space of time.

It may be mentioned that for all the above work the reaction was adjusted to -10.

Similar experiments have been carried out using ordinary brewer's yeast both fresh and dried instead of caseinogen. Solid medium made up in the ordinary way from undigested fresh yeast extract possesses small nutritive value and the medium obtained after a two days' tryptic digestion is by no means as good as that from a similar digestion of caseinogen.

The nutritive powder prepared from fresh yeast in a similar manner to that from caseinogen also yields a fair growth of *B. typhosus*.

Culture medium prepared from dried yeast behaves in a similar manner. Growths of *B. typhosus* obtained on solid media made up in the usual way from undigested yeast extract and from the products of a two days' tryptic hydrolysis are poor. On media prepared from the nutritive powder obtained from yeast, the best results were given by a 5% concentration but these were only as good as those from a 2% concentration of the powder prepared from caseinogen.

## SUMMARY AND CONCLUSIONS.

1. The preparation of a dry nutritive powder from caseinogen is described.

2. This powder does not require the addition either of peptone or extractives but may be made up into solid nutritive medium merely by the addition of agar and normal saline or tap water.

3. It is obvious, therefore, that it would be extremely useful for field workers both on account of its portability and the extreme ease with which it can be made into culture medium.

4. It is suggested that the name 'trypsinoids' be given to the powder.

5. Similar experiments with ordinary brewer's yeast fresh and dried are described but it would appear under the conditions tried that caseinogen yields the more nutritive powder of the two.

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# A SUMMARY OF RECENT OBSERVATIONS UPON THE ANOPHELES OF THE MIDDLE EAST.

BY

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THE area with which this paper is concerned, and which for want of a better term I have called the Middle East, is a large tract of desert or semi-desert country lying at the meeting place of the three continents of Europe, Asia, and Africa. It includes Asia Minor, Syria, Palestine, Arabia, Mesopotamia, Persia, Turkestan, Afghanistan, and Baluchistan. The total area is about twice that of India proper and not far short of the whole of Europe including Russia.

Of the anopheles of this relatively large tract, prior to the war, practically nothing was known.

In 1902 Cropper<sup>(1)</sup> in a paper on Malaria in Northern Palestine records from this country as identified by Theobald, *A. maculipennis* Meigen, *A. pseudopictus* Grassi, *A. superpictus* Grassi, and *A. pharænsis*. Theobald, *i.e.*, three of the four species commonly found in South Europe and one (the last) an African species.

In 1905 Patton<sup>(2)</sup> described five species of anopheles from the Aden Hinterland. These he considered new and named from the localities where they were found *A. arabiensis*, *A. d'thali*, *A. tibani*, *A. jehafi*, and *A. azriki*. The synonymy of these species remained however, for many years very obscure.

In 1911 Sub-Asst. Surgeon Rai Sahib Khazan Chand on behalf of the Malaria Bureau was permitted by the Director-General, Indian

Medical Service, to accompany the Sanitary Commissioner on a visit to Aden and to travel over a portion of the area from which Patton had recorded his species. Sub-Asst. Surgeon Khazan Chand brought back a very excellent collection of anopheles, establishing the fact that on the mainland of Arabia near Aden the common species were *A. culicifacies*. Giles and *A. rhodesiensis*. Theo. Included in the collection were also specimens of *A. costalis*. Theo. From this collection and other sources of information it was possible to give the synonymy of Patton's species as follows<sup>(3)</sup> :

<i>A. arabiensis</i>	=	<i>A. costalis</i> . Theo.
<i>A. d'thali</i>	=	<i>A. rhodesiensis</i> . Theo.
<i>A. tibani</i>	=	<i>A. pretoriensis</i> . Theo.
<i>A. jehafi</i>	=	<i>A. cinereus</i> . Theo.
<i>A. azriki</i>	=	<i>A. turkhudi</i> . Liston.

Considering the conditions under which Major Patton was working and the early date of the observations, the number of species found by him must be considered a matter of congratulation. The only species since added to his list has been *A. culicifacies*. Giles.

Shortly after the outbreak of the war Major Gill, I.M.S.<sup>(4)</sup>, whilst on active service in Muscat (1915) made a study of the anophelines of the locality and sent to India specimens of *A. rhodesiensis*, *A. culicifacies*, and a variety of *A. funestus*. Giles. He also records *A. cinereus*. Theo. As regards the south of Arabia, therefore, the anopheline fauna is definitely African.

In the same year (1915) specimens of anopheles were sent by Capt. Shortt, I.M.S., Lt.-Col. Perry, I.M.S., and Lt.-Col. Hehir, I.M.S., from Lower Mesopotamia. So far as I have been able to ascertain, these were the first mosquitos ever seen from this area. The species represented were *A. pulcherrimus*. Theo., *A. stephensi*. Theo., and *A. sinensis*. Wied.<sup>(3)</sup> A small species with unspotted wings thought to be new and named by the author *A. lukisii* was also found at Amara and at Accab on the Tigris in 1916.<sup>(5)</sup> This is distinct from a species at Busra, usually rare but at times abundant, resembling *A. maculipennis*, though with very faint markings on the wings and with eggs quite unlike those described for the latter species.

North of Baghdad Capt. Shortt, I.M.S., and the author found the species characteristic of the Lower Tigris and Euphrates give place to quite another set of species, the commonest being *A. superpictus* and

*A. maculipennis*. At Dohuk, north of Mosul, a single specimen of *A. bifurcatus*. Linn. was encountered. In this part of Mesopotamia also was found a specimen of *A. rhodesiensis*, shewing that this African species occurred though apparently rare. The European faunas of the anopheline fauna is continued at least as far east as the south shores of the Caspian as shewn by Major Christie, R.A.M.C.,\* who in 1918 visited this region and found *A. maculipennis* everywhere abundant.

In Palestine, Syria, and Cilicia the anopheline fauna, as recently determined, is also predominantly European in character. The commonest species as noted by Lt. Barraud†, entomologist to the Egyptian Force, in all these areas are *A. maculipennis*, *A. bifurcatus*, and *A. superpictus*. *A. culicifacies*. Giles, an African and Indian species, is, however, recorded from Lake Tiberias by Prof. Annandale (1915)(6) and *A. pharænsis* previously recorded by Cropper is also typically African. Lt. Barraud also informs me that *A. turkhudi*. Liston occurs on the Palestine coast, especially in brackish water, and has very kindly given me a number of references to reports during the war in which other species as noted below have been reported. Some at least of these species must be considered as still requiring confirmation.

*A. mauritanus*. Grand- Found uncommonly in Palestine. E. E. pre. Austen(7).

*A. algeriensis*. Theo. . . Mentioned by E. E. Austen(7) as found in Palestine.

*A. plumbeus*. Haliday . . One specimen from Katma, Cilicia, Lt.-Col. Lelean, R.A.M.C., and Lt. Watts, I.M.S.

*A. immaculatus*. Theo. . . One specimen from Ain Sofar, 4,500 feet, Lebanon Mts., Lelean and Watts.

Passing to the east of the area we approach the frontiers of India. On the North-West Frontier early work by a number of observers, of whom we may mention Col. Nurse, R.A.M.C., Major Davys, I.M.S., Capt. Sinton, I.M.S., and Major Dunbar-Walker, R.A.M.C., had shewn that in addition to many Indian species there existed at least one not recorded from anywhere else in India. This species formerly known to Indian workers as *A. narsei*. Theo. is now known to be synonymous with *A. superpictus*. At Quetta (5,000 ft.) this is the common anopheline. Capt. Sinton informs me that in addition he finds fairly generally

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\* Private communication.

† Private communication. (Cf. also Austen(7).

distributed another non-Indian species, *A. rhodesiensis*. The specimens sent by this officer shew this to be correct.

Quite recently Capt. Sinton has sent me a most interesting and valuable collection of anopheles from Eastern Persia and Turkestan. *A. superpictus* is represented by three specimens, two from Nistafun in Northern Persia and one from beyond Merv in Turkestan. The predominant species, however, represented by numerous specimens from a large number of localities in East Persia (from the Baluchistan border to approaching the Caspian) is *A. turkhudi*.

Our knowledge, then, of the distribution of species in the Middle East may be summed up as follows.

*A. pulcherrimus*. Theo. appears to be a species whose diffusion centre (Zoocentre) lies within the area. In India this species has long been known as occurring more or less sporadically in the Punjab. It is common in Sind and large numbers of specimens are sent to the Bureau from the Indus. In Mesopotamia *A. pulcherrimus* occurs ubiquitously and in enormous numbers, being easily the dominant anopheline of Lower Mesopotamia. The Syrian and Arabian deserts seem to form the western limit of distribution, for in Palestine *A. pulcherrimus* does not occur but its near relative the African *A. pharansis*.\*

*A. superpictus*. Grassi ranges from Baluchistan through the highlands of Persia to Asia Minor and the Balkans. It occurs also in North Africa in Algeria, Tunis, etc. Its range into Central Asia is unknown. The distribution is neither Holarctic, African, nor Oriental, but in some degree special to the area under discussion (Irano-Ottoman).

*A. turkhudi*. Liston also has a most interesting distribution. It occurs in Spain (*A. hispaniola*); under the name of *A. chaudiyei* it is well known in the Sahara, where it appears characteristic of the Oases (Beni-Ounif, Wady Rir, El Oued); it occurs also in Egypt (*A. multicolor*), in Arabia (Aden Hinterland), and in Palestine. It has now been shewn to extend through the deserts of East Persia and thence into India where it becomes rare away from the north-western portions. The distribution of *A. turkhudi* is therefore also to some extent special to our area. Whilst *A. superpictus* occurs in a belt-like zone mainly north of the Mediterranean and is associated especially with hilly and submontane conditions, *A. turkhudi* follows a parallel course to the south and is associated with

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\* Vassiliev<sup>(8)</sup> also gives *A. pulcherrimus* as the dominant anopheline in Russian Turkestan.



## 714 *Observations upon the Anopheles of the Middle East.*

the zone of great deserts (Sahara, Arabia, Seistan, N. W. India). A suitable name for this type of distribution would appear to be Saharan.

The remaining species that have been mentioned are extensions from outlying faunas as follows :—

Holarctic.	Oriental.	African.
<i>A. maculipennis.</i>	<i>A. stephensii.</i>	<i>A. costalis.</i>
<i>A. bifurcatus.</i>	<i>A. sinensis.</i>	<i>A. rhodesiensis.</i>
<i>A. plumbeus.</i>		<i>A. pretoriensis.</i>
		<i>A. cinereus.</i>
		<i>A. culicifacies.</i>

*A. maculipennis* occurs as a hill species to within 50 miles of Baghdad, a single typical specimen having been taken as far south as Amara on the Tigris (Col. Wenyon, R.A.M.C.). There is also, as previously noted, a variety of this species or a distinct but related species found about Busra.

*A. bifurcatus* apparently does not extend so far as its European congener, for though *A. bifurcatus* is recorded as common in Palestine only a single specimen has been found in Mesopotamia and this far to the north.

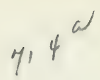
*A. plumbeus* occurs in Kashmir and the North-West Himalayas and may very possibly occur in the Caucasus, Taurus, etc.

The oriental species *A. stephensii* found in all the regions of India proper and noticeably in Sind extends up the rivers of Mesopotamia becoming more and more patchy in its distribution. The Syrian desert here again seems to form the western limit of distribution of a species, since further west no species which could possibly be *A. stephensii* is known.

*A. pseudopictus* is one of a series of closely related sub-species or varieties of *A. sinensis*, which belts the Old World from China and Malay to Europe.

The African fauna extends at least into Southern Arabia as represented by the very typically African species *A. costalis*. *A. rhodesiensis* must be considered an African form, but it has a wide distribution eastwards and to the north. *A. culicifacies* occurs throughout India proper and also in Africa, these two areas of distribution being apparently connected only by a narrow neck across southern Arabia. *A. culicifacies* has never been found in Mesopotamia, the absence of a species which in India is everywhere associated with canal irrigation being one of the most important epidemiological features in the

7, 4<sup>a</sup>



7, 4<sup>a</sup>

7, 4<sup>a</sup>

The map illustrates the distribution of the Holarctic and Oriental biogeographic realms in the Eastern Hemisphere. The Holarctic realm, indicated by diagonal hatching, covers the Arctic region, the Black Sea, the Mediterranean Sea, and parts of the Middle East and North Africa. The Oriental realm, indicated by cross-hatching, covers the Indian subcontinent, the Malay Archipelago, and parts of Southeast Asia. The map also shows the Persian Gulf, the Red Sea, and the Arabian Sea. The borders of Afghanistan, Persia, and Arabia are marked. The legend in the bottom right corner provides the key for the shading.

Explanation

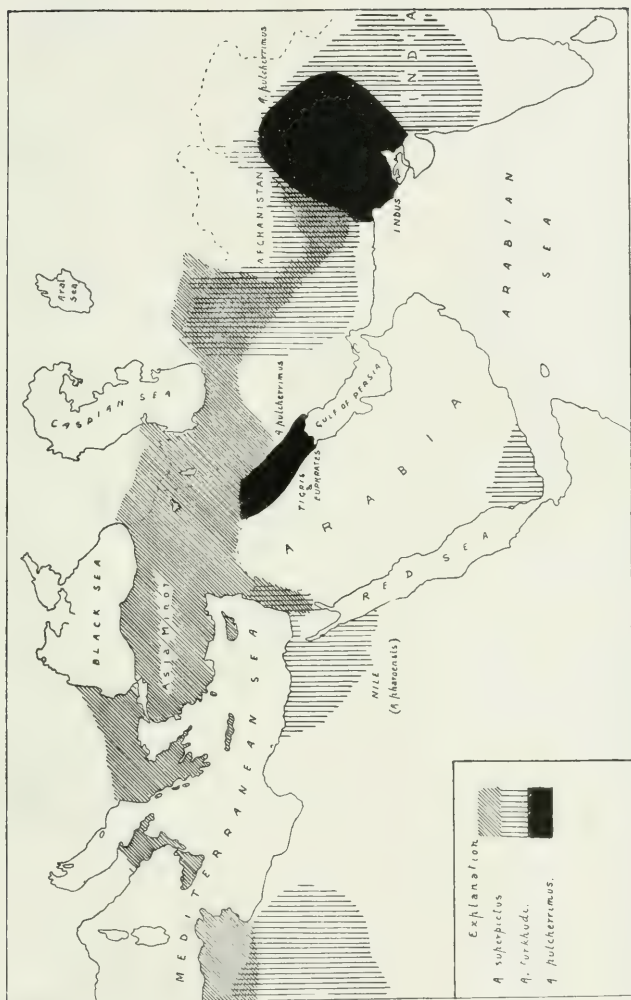
Holarctic

Oriental

Map illustrating extension of Holarctic and Oriental anopheline faunas.

**S. R. CHRISTOPHERS.—A Summary of Recent Observations upon the Anopheles of the Middle East.**

MAP III.



Map illustrating distribution of species whose centre of diffusion lies in the region intermediate between Holarctic, Ethiopian, and Oriental regions.

NOTE.—*A. pulcherrimus* should also be shown in Russian Turkestan.

S. R. CHRISTOPHERS.—A Summary of Recent Observations upon the Anopheles of the Middle East.



Tigris and Euphrates basin where large canal schemes will be necessary for the development of the country.

Taken collectively the Holarctic species extend to a line drawn through Palestine and across Mesopotamia to the Caspian. The African fauna is typically represented in Arabia and extends in a modified form far to the east. Species belonging to the Indian fauna do not pass the line of the N. W. Frontier except in the case of *A. stephensii* and the widely distributed *A. sinensis*. There are species that are neither Holarctic, Ethiopian, nor Oriental, but special to intermediate areas, Irano-Ottaman and Saharan.

As regards the reasons for these distributions, they must be sought in part at least in the various land changes and climatic alterations which have taken place in geologic times. There is some reason to believe that the distribution of the chief groups of anopheline types took place very early, possibly as far back as the mesozoic period. In respect to the area under discussion the final distribution of species or their local evolution must have taken place much later, since during Eocene times a great part of the Middle East formed the floor of the great Central Mediterranean Ocean. It is most probable that in the main the anopheline fauna of the Middle East reached its present distribution in the Miocene period, when important changes in the general land fauna of the area took place.

As regards the mammalian fauna, according to Suess<sup>(9)</sup>, there was developed in the Miocene period (2nd Mediterranean stage) a fauna with common features, which extended from Europe to the Far East and which, since the existing fauna of Malay is of this type, is termed Malayan.

Following the Malayan there appeared, associated with the great fresh water lakes of the Pontic Stage (3rd Mediterranean stage), a second type of fauna which since it resembles that at present found on the African continent is known as African. This African fauna extended much beyond the present continent of Africa and over a great part of India, replacing the Malayan fauna except from certain areas, e.g., Malabar.

Finally in Pliocene times there was an extension southwards of the Holarctic fauna.

The distribution of species of anopheles in the Middle East is on the whole in close accord with these general changes in the mammalian fauna.

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# MALARIA OF MONKEYS.

AT THE FOOT OF THE NILGIRIS.

*During the months of May and June, 1919.*

BY

LIEUT.-COL. C. DONOVAN, I.M.S.

[Received for publication, January, 13, 1920.]

THE object of this investigation was to decide if the common monkeys (*Macacus sinicus*, and *Presbytis priamus*) frequenting the very malarious regions at the foot of the Nilgiris suffered from malaria, that is, if they were infected by the red-blood parasites, *Plasmodium* and *Lacerania*; and if the find was positive, whether these parasites were closely akin or identical with the species in man. Then they might be taken as reservoirs of the malarial parasites in regions uninhabited by human beings: bringing to mind the analogous condition of affairs between the African antelopes assumed to act as reservoirs of the sleeping sickness trypanosomes.

Statements are commonly made by planters and shikaris that malaria can be contracted in jungles where no human beings dwell, or which are but very rarely frequented by them. Several such places were mentioned to me at the foot of the hills under question. The reasons given appeared plausible and it interested me to prove the correctness of these assertions by undertaking the examination of the monkeys in the localities falling under the above category.

I append a rough sketch map showing the localities marked in red dots; the parts visited by me in linked spots in the same colour. The map is painted in colours giving the different contours of this mountain range. The portions given in light mauve corresponding to 3,000 to 2,000 ft. elevation and downwards are the most malarious. It may be stated, speaking generally, that all round the foot of the Nilgiris malaria

is ripe. To mention the spots separately the following are of special note.

*Kallar*, at the foot of the Coonoor ghat, is notorious for its severe form of fever. There is a frequent coming and going of country carts at all hours of the day and night, and a few ryots sleep in huts to tend the luxuriant areca-nut palms, for which this locality is famous. Hence this place was not suitable for carrying out experiments with monkeys, as man was present and could supply the parasite.

The forest country between *Gudulur* and *Teppikadu* in the Nilgiri-Wynaad appeared more suitable. The area was extensive and presumably no human beings frequented some of the more remote spots. I selected the locality for several reasons as will be mentioned hereafter.

The Bhavani Valley is another noted spot for these fevers and, my informants were positive, unfrequented by human beings in many places. I was unable to give the tract any attention on account of its inaccessibility.

*Sigur Ghat and the road to Teppikadu.*—Here there is no doubt of malaria of human origin. There are houses scattered about in this area.

*Moyar Valley.*—An ideal spot, if any, for malaria of animals, as it is very rarely that it is frequented by man. I regret that I found a sojourn in this wished-for paradise for research barred on account of the difficulty of obtaining provisions and the great heat. At the time of my visit it was swarming with wild elephants, whose acquaintance was undesirable with a small bore rifle. I was warned by the Forest Officer to give the forest here a wide berth.

Other regions not so suitable were the Nilambar Ghat, a spot on the northern side looking into the Moyar Valley, and two others on the eastern face of the Nilgiris. All these places are marked in the map accompanying this paper.

To return to the country between Gudulur and Teppikadu in the Nilgiri-Wynaad: this looked a most suitable spot and took up most of the time at my disposal. A road ran through the forest and monkeys were numerous. The animals were picked off with a 300 high velocity rifle with soft nose bullets. If struck anywhere in the body, death was instantaneous. Apparatus for taking blood smears and performing an autopsy were near at hand. The thoracic and abdominal viscera were quickly and fully examined and their condition noted. Whenever possible male adults were selected, but occasionally a female was bagged.

The animals were very quick and agile in getting out of rifle range amongst the branches and snapshots had to be taken of many of them. Hence picking out the males was not always possible. The healthy condition of the monkeys procured was remarkable: their viscera were in a perfect normal condition. Most of them harboured ticks (species not determined) with which these jungles abounded.

This tract was asserted to be free from human inhabitants, but in my peregrinations through it I met with several jungle people wandering about in the remotest parts and some of them had temporary huts therein. The supposition of these jungles being man-free, so to speak, was incorrect, and the malaria here was undoubtedly of human origin. The examination of the blood of some human beings shewed the presence of either active or passive malarial infection. The human intervention was disappointing and showed with what caution all statements by laymen are to be accepted. It might then be asserted that 'no human beings no malaria,' but further and fuller experiments are necessary before making this dogmatic assertion.

The monkeys examined were 76 specimens of *Macacus sinicus*, the common species all over South India, and 10 langurs (*Presbytis priamus*), the peninsula species or rather variety of the Indian Langur. With a few exceptions all were males. None of these, 86 in number, contained a malarial parasite of any kind. That is, there was an absence of the genera *Plasmodium* and *Laverania*, commonly known as the benign tertian, quartan and malignant tertian kinds. The rare Nilgiri monkeys, *Macacus silenus* and *Presbytis johni*, were not molested in any way, as these animals are becoming rare and of local distribution.

Although I have detected neither *Plasmodium* nor *Laverania* in *Macacus sinicus* or *Presbytis priamus*, I have found species of the genus *Plasmodium* in the following kinds of monkeys; in the orang-outang, *Plasmodium pitheci*; in *Cercopithecus* sps.?, *Plasmodium kochi*; and in *Macacus cynomolgus* (closely akin to *Macacus sinicus*), *Plasmodium cynomolgi*.

Although the find in the monkey at the foot of the Nilgiris gave a negative result, I was struck by the frequent infection of the Malabar squirrel—a denizen of these parts—with *Plasmodium ratufa*, sps. nov. a parasite very alike *P. vivax* of man. The gametocytes predominated and in a few, strange to say, the female gametocytes exceeded in number the male forms. The squirrels—*Ratufa indica malabarica*—were very

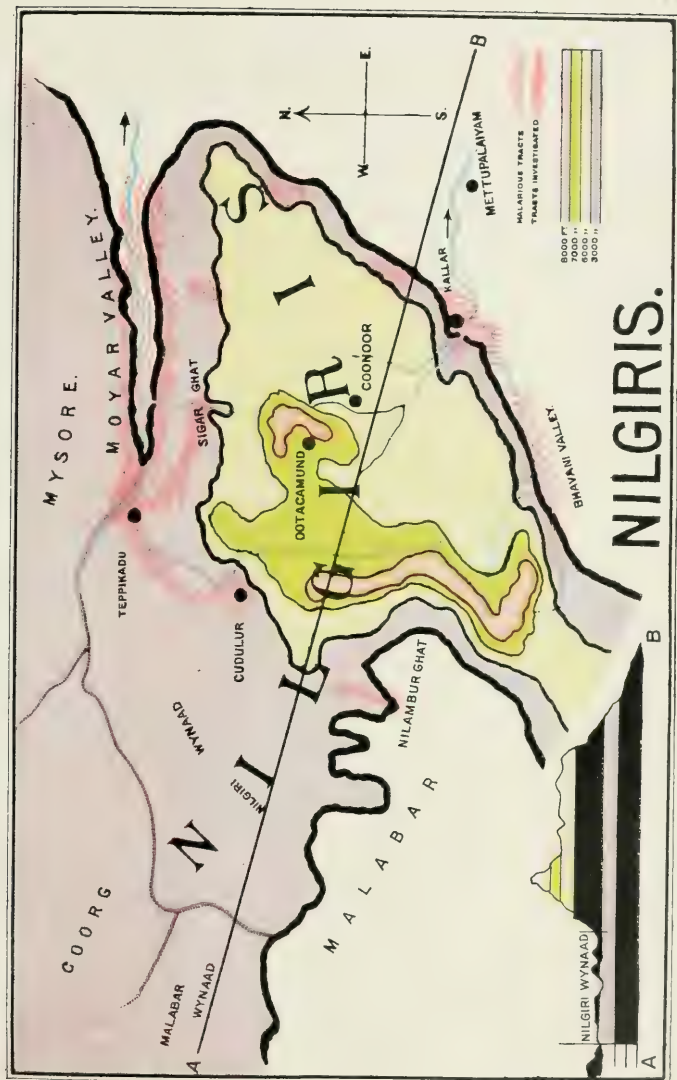
healthy and active and showed no signs of illness. Out of 8 procured, 7 were infected with this species of the malarial parasite. It was a pity to kill a larger number of these handsome animals. It may be stated that Vassal found a similar if not identical parasite in a squirrel (*Sciurus corseanus*) in Annam, *vide* Annales de l'Institut Pasteur, Tomes 19 and 21.

Up to the present time no malarial parasite similar to the malignant tertian kind and known as *Laverania* has been found in animals: it is special to man. The malarial parasites in mammals other than man, for instance monkeys, bats and squirrels, all are relegated to the genus *Plasmodium*. Consequently it may be stated that the malignant form of malaria is of human origin only. In relating my experience of these investigations at the foot of the Nilgiris the other day to Manson, he suggested the examination of cave-dwelling bats. Primitive man being a cave-dweller might have contracted the malaria from his co-dwellers the bats. I have examined bats and found blood parasites in them, referable more to *Peroplasma* than *Plasmodium*, though I have found the latter parasite in Fijian flying fox and Mackie has found it in the flying fox of Assam. The bats I have so far examined were not cave-dwellers, so this suggestion of Manson, our doyen of tropical diseases, is worthy of consideration for future workers.

Malignant tertian fevers were very common during my stay in the Gudalur-Teppekalu tract and the people here were dying every day from severe infections of this malarial. My daughter, who accompanied me and helped in my investigations, contracted malignant malaria at Gudalur and suffered from several very smart attacks.

To sum up it may be stated in conclusion that:—

1. No malarial parasites were found in 76 *Macacus sinensis* and 10 *Presbytis prasinus*.
2. The Malabar squirrel (*Ratufa indica malabarica*) commonly harbours a *Plasmodium* very like *P. vivax* of man.
3. The so-called uninhabited malarious tracts at the foot of the Nilgiris commonly have wild tribes wandering through and living in these regions.
4. The Malignant tertian parasite (*Laverania malarix*) has not so far been found in any animals, with the exception of man, and consequently is essentially a human parasite.





5. Cave-dwelling bats may harbour a malignant tertian parasite: an examination of such bats is needed.

NOTE. A letter received from Lieutenant Colonel Donovan dated 8th January, 1920 contains the following sentence:—

‘ Since sending in, on the 18th December, 1919, my report on the malaria of monkeys at the foot of the Nilgiris, a slide has been sent me of the blood of *Macacus sinicus* containing a *Plasmodium*. The animal was procured on the north side of the Nilgiris overlooking the Moyar Valley at the altitude of 4,000 feet. The parasite is morphologically identical with that found in the blood of *Macacus cynomolgus*. A further and more extended scrutiny of the blood of the monkeys in this area is indicated.’



THE CORRELATION BETWEEN THE CHEMICAL COMPOSITION OF ANTHELMINTICS AND THEIR THERAPEUTIC VALUES IN CONNECTION WITH THE HOOK-WORM INQUIRY IN THE MADRAS PRESIDENCY.

BY

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AND

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[Received for publication, April 15, 1920.]

V. OLEUM CAJUPUTI.

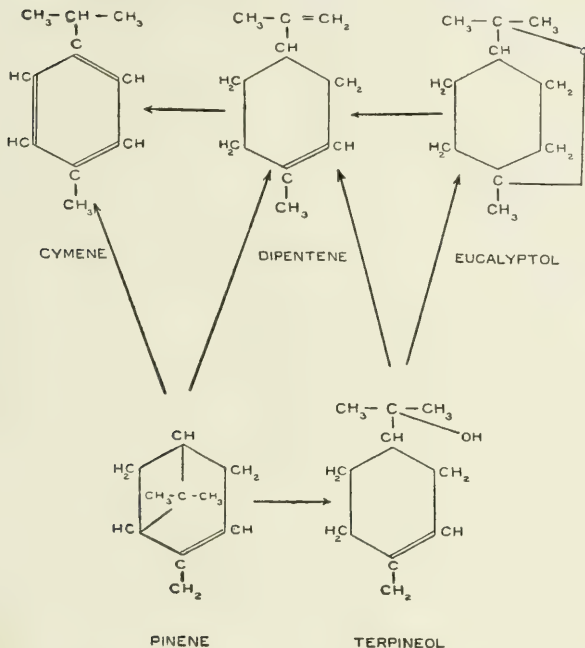
HISTORY.

*Gomenol*, a volatile oil obtained from the leaves of *Melaleuca Leucadendron* var. *minor* (= *M. viridiflora*), was used in 1910 by Brimont<sup>(1)</sup> as an anthelmintic in ankylostomiasis in doses of five minims. In 1914, Blin<sup>(6)</sup> reported from the gold mines of Moroni in Guiana that an indigenous drug, *Melaleuca viridiflora*, had given excellent results in mild cases of uncinariasis.

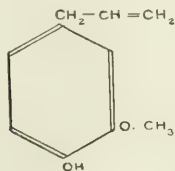
COMPOSITION AND PROPERTIES.

Oil of cajuput is distilled from the fresh leaves and twigs of several varieties of *Melaleuca Leucadendron*. It is a green limpid oil having a camphoraceous odour, and a warm, pungent taste, with specific gravity varying from 0.914 to 0.930, and boiling point 175–252 C. Its chief constituent, *cajuputol*, has the formula  $C_{10}H_{18}O$ , and, as shown by Wallach<sup>(1)</sup> and by Jahns<sup>(2)</sup>, is identical with *cineol* or *eucalyptol* from *Eucalyptus globulus*. A second constituent of the formula  $C_{10}H_{18}O$  is the solid *terpinol*, which is present both in the free state and as acetic ester. A small amount of pinene and traces of aldehydes have also been found<sup>(3)</sup>.

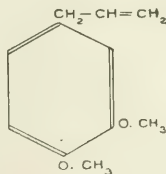
## Relationships of the constituents of oil of cajuput.



Schimmel & Co.<sup>(7)</sup> report that of two oils obtained from *Melaleuca Leucadendron* var. one was found to contain only a small quantity of eucalyptol, whilst the other contained 78 per cent methyleugenol and no eucalyptol.



EUGENOL



METHYLEUGENOL

The first sample of oil we used—Oil I—was a fluid transparent oil, of a fine green colour tinged with yellowish brown. It gave the following results: specific gravity at 29°C. = 0.8826; refractive index at 31°C. = 1.475; solubility in 90 per cent alcohol = 1:0.5; acid number = 0; ester number = 10.45; acetyl ester number = 29.1; boiling point = 163°–250°C. It contained only a very small amount of eucalyptol. The low specific gravity and boiling point, and the abnormal solubility in alcohol, led us to suspect the purity of the sample, which was accordingly fractionated. As much as 86 per cent of the oil distilled over below 175°C. and was found to consist of oil of turpentine together with a small quantity of oil of pine. The amount of eucalyptol calculated from the cajuput fraction corresponded to 2.1 per cent of the original oil.

The second sample—Oil II—was a transparent light brown oil with a greenish tint, and boiled at 173°–250°. It had:—specific gravity at 28°C. = 0.9367; acid number = 4.5; ester number = 20.7; acetyl ester number = 58.1; refractive index at 29°C. = 1.4682; optical rotation at 29° = +8°. It dissolved in 1 and more volumes of 80 per cent alcohol and contained 40 per cent eucalyptol.

A third sample—Oil III—showed an emerald green colour. It boiled at 165°–210°C. and gave the following abnormal results: specific gravity at 29°C. = 0.888; optical rotation at 31° = +20°. The portion distilling below 175°C. represented 75 per cent of the oil and was found to consist of oil of turpentine. Dextro camphor was identified by means of its oxime in the fractions coming over between 175° and 193°C. No eucalyptol could be found.

Cajuput oil when swallowed produces a sense of heat, with an increased fulness and frequency of pulse, and in some instances excites profuse perspiration.

#### ABSORPTION AND ELIMINATION.

The urines were generally acid and had no characteristic odour or colour. Their volume and density were normal. No smell was perceived after acidifying with hydrochloric acid and heating. According to Hamalainen<sup>(5)</sup>, eucalyptol is oxidised to cineolic acid before conjugating with glycuronic acid. No albuminuria occurred.

#### ANTHELMINTIC VALUE.

Healthy male prisoners were treated with increasing doses of cajuput oil shaken with gum acacia emulsion and administered in two portions at half an hour's interval. This treatment was preceded by an overnight dose of Epsom salts and followed by another dose two hours after. No

food was allowed until the bowels had moved. The limit of tolerance was reached with a dose of 60 minims as noted from the very marked giddiness observed in two cases.

The total hookworm content was determined in the usual routine way using 60 grains thymol as a subsequent standard treatment.

The results showed that :—

1. Cajuput oil is toxic in doses of 60 minims which produce giddiness.

2. In a series of 58 cases treated with varying dosages of oil the number of hookworms removed was 78 out of a total of 1,562. The worms were mostly dead and contorted in shape.

3. No ascarids were removed though round worms were present in 18 cases and whipworms in 15.

*Number of hookworms removed by one test treatment of Cajuput Oil.*

Experiment number.	TEST TREATMENT.		Number of cases treated.	HOOKWORMS REMOVED.				PERCENTAGE OF HOOKWORMS REMOVED WITH A TEST TREATMENT.			
	Drug used.	Dosage.		A. duodenale.	N. americanus.	A. duodenale and N. americanus.		A. duodenale.	N. americanus.	A. duodenale and N. americanus.	
1	Cajuput Oil No. 1.	5 to 15 minims.	6	Test treatment.	0	1	1	0.0	0.9	0.8	
				Subsequent treatments.	7	110	117				
				Total hookworms.	7	111	118				
2	Do.	18 to 30 minims.	6	Test treatment.	2	3	5	20.0	0.8	1.1	
				Subsequent treatments.	8	336	344				
				Total hookworms.	10	339	349				
3	Do.	33 to 40 minims.	1	Test treatment.	0	1	1	0.0	0.3	0.3	
				Subsequent treatments.	8	300	308				
				Total hookworms.	8	301	309				

Number of hookworms removed by one test treatment of *Cajuput Oil*.

Experiment number.	TEST TREATMENT.		Number of cases treated.	HOOKWORMS REMOVED.			PERCENTAGE OF HOOKWORMS REMOVED WITH A TEST TREATMENT.		
	Drug used.	Dosage.		A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.
4	Cajuput Oil No. II.	30 to 35 minims.	2	Test treatment.	0	4	4	0.0	4.1
				Subsequent treatments.	1	93	94		
				Total hookworms.	1	97	98		
				Test treatment.	1	19	20	11.1	13.2
5	Do.	40 minims.	12	Subsequent treatments.	8	125	133		
				Total hookworms.	9	144	153		
				Test treatment.	1	16	17	25.0	14.2
				Subsequent treatments.	3	96	99		
				Total hookworms.	4	112	116		
				Test treatment.	0	30	30	0.0	7.4
				Subsequent treatments.	17	372	389		
				Total hookworms.	17	402	419		

Oil of cajuput cannot be recommended as an anthelmintic.

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DRACONTIASIS IN ANIMALS ;  
WITH NOTES ON  
A CASE OF GUINEA-WORM IN A COBRA.

BY

D. A. TURKHUDD, M.B., C.M., EDIN.

[Received for publication, March 18, 1920.]

*Dracontiasis in domesticated animals.*—The occurrence of dracontiasis among domesticated animals is not unknown and a number of investigators have shown that the disease may be met with in dogs, horses and cattle (<sup>1</sup>); even in India it appears to be somewhat rare amongst them.

During the course of investigations conducted in the Bombay Bacteriological Laboratory in connection with guinea-worm disease the Director and the staff of the Laboratory visited a number of villages in the Bombay Presidency in which the disease was known to be widely prevalent among human beings; but in these excursions they came across no cases showing the presence of the worm in any of the village animals such as cats, dogs, goats, horses and cattle, although these animals always drank the same water as the inhabitants of the infected villages. To the villagers themselves these animals were not known to suffer from guinea-worm.(<sup>2</sup>)

*Dogs.*—The presence of guinea-worm disease in dogs in India has been reported by a few observers. Smyttan (<sup>3</sup>) has described the case of a dog which, 'after being lamed for a few days,' was seen 'to pull a guinea-worm out of the lame leg with his teeth.' Forbes (<sup>4</sup>) has recorded the case of a guinea-worm extracted from the neck of a dog. Gaiger (<sup>5</sup>) has reported five cases of *Filaria medinensis* in dogs. Two of these occurred in the Dog Hospital, Punjab Veterinary College, while the other three were detected in the Veterinary Hospital, Simla, by Gulam Hussein, the

Veterinary Assistant in charge. Drawings of an adult worm and embryos are given, and Gaiger states that 'in all cases the worms were identical with the human parasite and contained a white fluid in which were the coiled up embryos.'

The Museum of the Madras Veterinary College has a specimen of a guinea-worm removed from a dog. This specimen, which was sent to the Parel Laboratory for inspection, is not an entire worm but only a portion of the tail end. In its preserved condition it is 2 mm. in width with the characteristic curved tail, and closely resembles the human guinea-worm. The history of the case obtained through the courtesy of Principal Aitchison of the College, is as follows :—<sup>(6)</sup> 'The subject was a dog, aged three years, brought for treatment to the Veterinary Hospital at Adoni, a town in the district of Bellary in this (Madras) Presidency in which human dracontiasis is very common among the people. The dog prior to its admission into the hospital was reported by the owner to have become off-feed with attacks of obstinate vomiting, restlessness and utricular eruptions all over the body. A fortnight after the occurrence of the symptoms the owner noticed the appearance of a swelling on its hind leg to which he applied a poultice of mustard and egg thinking it to be due to a sting of an insect. As the swelling was persistent and caused considerable pain, the animal was taken to the Veterinary Assistant who noticed a vesicle at the centre of the swelling. On opening it a small quantity of straw coloured fluid escaped bringing into view the head of the worm; this was seized and the worm slowly extracted. With a week's treatment at the hospital the wound healed and the dog was discharged, and up to the present has not shown any further signs of ill health. No other cases have been reported among dogs from the same neighbourhood.'

In the year 1917 experiments were conducted at the Parel Laboratory to produce guinea-worm disease in dogs by feeding them on infected cyclops; but the experiments proved unsuccessful.

*Horses.*—The occurrence of dracontiasis among horses in India has also been reported. Forbes <sup>(7)</sup> has described a case of guinea-worm in a country bred pony at Dharwar in Bombay Presidency. The worm protruded from a swelling near the right hind fetlock and was of the usual size. He writes 'I examined it before and after extraction and could perceive no difference in any respect from the human *Dracunculus*.'

Clackson <sup>(8)</sup> found a guinea-worm in an Australian waler at Kotagherry on the Nilgiri Hills.



Cobbold (<sup>9</sup>) who states that 'the occurrence of *D. mediusensis* in the horse is extremely rare,' has reported the case of a guinea-worm extracted from a pony at Secunderabad.

Batliwala (<sup>10</sup>) in a paper read at a meeting of the Bombay Veterinary Association, has described a case of guinea-worm in an Arab gelding in Burma. 'The worm' he writes 'looked very much like the guinea-worm which is very common in man in Katywar.'

On the 16th August, 1914, Mr. V. L. Bhawe, B.Sc. (Bombay), sent to this Laboratory a specimen of a guinea-worm extracted from a pony at Thana in Bombay Presidency. This case occurred during the course of the investigation conducted by the Director and staff of the Laboratory, but it was not brought to their notice at the time. Unfortunately the specimen when received had already become partially decomposed, and upon examination it was found to be only a fragment of a worm; it was 10 cm. in length and 1 mm. in breadth and the extremity was provided with a curved hook-like tail.

*Cattle.*—Blanchard states that the disease was known in 15th century Arabic medicine to occur among cattle (<sup>11</sup>). Bartet (<sup>12</sup>) on the authority of Heckenroth asserts that the disease is met with among cattle in West Africa. But there are no records showing the existence of dracontiasis among the Indian cattle.

*Wild animals.*—As regards the presence of dracontiasis among wild animals the records are very scanty indeed. A few cases, however, have been described showing that guinea-worm is not unknown among some of the wild animals in Africa.

According to Raillet, (<sup>13</sup>) Piot has recorded guinea-worm in a jackal killed in Upper Egypt and also in an Egyptian wolf.

Valenciennes (<sup>14</sup>) found a number of guinea-worms coiled up in the connective tissues under the skin of a hunting leopard (*Felis jubata*) from Kordofan, and Cazalbou (<sup>15</sup>) came across similar worms in a monkey (*Cercopithecus callitrichus*).

Leiper (<sup>16</sup>) has recorded an interesting case of dracontiasis in a leopard shot by Mr. Charles Grey at Broken Hill in N. W. Rhodesia, a part of Africa where guinea-worm disease is not known. On skinning the animal a worm was noticed by Mr. Grey to be sticking out of the wound in the shoulder. Leiper examined this worm, both extremities of which were unfortunately missing, and found that it resembled exactly the guinea-worm found in human beings on the West coast of Africa. In appearance it was white and glistening, 45 cm. long and 1.5 mm. broad.

The body was filled by a single uterine tube crowded with embryos which corresponded exactly in size and structure with those of *Dracunculus medinensis* from man.

The records showing the presence of guinea-worm among the wild animals of India are extremely scanty. Mr. E. Brooke-Fox (<sup>17</sup>) of Junagadh in Kathiawar has described the following case:—Early in last March (1913) I shot a chinkara (*Gazella bennetti*) buck: the first shot struck him rather far back, the second through the shoulder; he was in good condition but the coat was staring and patchy. On examining him closely I noticed that the blood flowing from the wounds contained half a dozen thread-like semi-transparent worms varying in length from 2 to 5 inches all alive and vigorous. The animal was "hallaed" and eaten by the Mohammedan forest guards. In Junagadh State—particularly in the South—guinea-worm is a common complaint amongst all classes, occurring chiefly in the families of those who draw their drinking water from step-wells or "Vavs." Dr. Dave of Junagadh tells me that from my description the chinkara was undoubtedly suffering from guinea-worm. This fact may be of interest to the medical members.

From the description given the worms appear to be too short to be guinea-worms. The specimens evidently were not actually examined by the Doctor.

*Wild animals in captivity.* About twelve years ago a guinea-worm was detected in a leopard sent to the Bombay Bacteriological Laboratory from the Victoria Gardens for post-mortem examination. The specimen unfortunately was not preserved.

In February, 1919, the Superintendent of the Victoria Gardens, Bombay, (<sup>18</sup>) sent to this Laboratory a specimen of a guinea-worm extracted from an abscess on the thigh of an Arabian baboon (*Cynocephalus hamadryas*). The worm is entire, 92 cm. long and 1.5 mm. in diameter and resembles a human guinea-worm in every respect. The previous history of the animal could not be ascertained in a satisfactory manner.

No other cases of guinea-worm among the wild animals in this country appear to have been placed on record.

The guinea-worm which is about to be described was found in a cobra in the Bombay Bacteriological Laboratory: the case is unique because of the occurrence of the worm in a poikilothermic animal. The cobra, a yellow spectacled *Naja tripudians*, had been purchased locally on the 8th of July, 1915.



PLATE LXV.



FIG. 1.

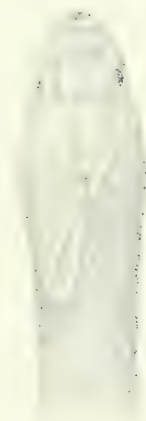


FIG. 2.

On the 10th of February, 1916, *i.e.*, seven months after the cobra had remained in captivity in the Laboratory, while venom was being extracted from it, a loop of a worm about 1 mm. thick was noticed to be protruding from a slight swelling on the head of the snake; the loop was about 4 cm. in length, cylindrical and glistening white in colour. The swelling was situated immediately behind the parietal shields; it was circular, about 1.5 cm. in diameter, slightly raised above the adjoining scales, and involved 6 or 7 of the vertebrae and costals immediately behind the parietals (Plate LXV, Fig. No. 1). There was a small round opening in the centre of the swelling through which the loop was seen to protrude; the opening itself was situated in the cuticle between the scales. Plate LXV, Fig. No. 2, gives an idea of the worm as it was first seen. Unfortunately during the manipulations necessary for photographing the animal, the loop broke. The broken worm was afterwards teased out in water and when examined under the microscope it showed a central uterine tube containing active embryos somewhat smaller than, but otherwise closely resembling, those of the human guinea-worm. The cobra was subsequently kept under special observation. From the 11th to 14th the animal appeared to be very irritable, the swelling on the head remained the same but no worm could be seen protruding from it.

On the 15th the snake was killed under chloroform; ice was then applied to the head and a small quantity of whitish fluid was seen to exude from the opening in the swelling; this opening could be plainly seen after removing the scales. The extruded fluid was sucked up in a pipette and added to a small quantity of water in a test tube; it was full of embryos which were sluggish at first but soon became quite lively and were seen swimming about in the water exactly like the embryos of a human guinea-worm; but even to the naked eye the difference in size was noticeable.

The cobra was then dissected. The dissection was commenced at the opening in the swelling; the worm was traced backwards and found to be lying between the muscles on the back, parallel to and along the right side of the spine. The anterior extremity was missing and when about 12 cm. of the worm had been dissected out, it was found to terminate in a short curved tail. The worm was 1 mm. in breadth, but in all other respects resembled the human guinea-worm.

The accompanying coloured drawing (Plate LXVI, Fig. No. 3) shows the situation of the worm; it had however shrunk a little by the time the sketch was completed.

The measurements of the living embryos obtained from this cobra guinea-worm were as follows :—Length 0·3 mm.; breadth (at the broadest part) 15–20 $\mu$ . From these measurements it will be seen that the embryos are much smaller than those of the human guinea-worm. The latter when examined in this Laboratory showed the following measurements : Length 0·6 mm.; breadth (at the broadest part) 20–30 $\mu$ —dimensions which coincide with those given by various observers.\*

On the same day (15th February), some of these embryos were added to water containing cyclops, while a few were allowed to remain in plain water and kept at room temperature; the latter, however, were all found dead after three days. The examination of the cyclops twenty-four hours afterwards showed that out of twelve cyclops ten were infected, *i.e.*, 83 per cent. showed infection. On the 20th February, out of 42 cyclops examined, 15 were found infected; the percentage of infection had gone down to 35·5. On the 29th February, 150 cyclops containing living cobra guinea-worm embryos were isolated from the stock. These cyclops had been fed on the 15th, consequently the embryos had undergone a fortnight's development within them. The guinea-worm embryos dissected out of cyclops on this day gave the following measurements : Length 0·6 mm.; breadth 30  $\mu$ , *i.e.*, they had nearly doubled in length and breadth during the period. These 150 infected cyclops were then divided into 6 lots of 25 each, and six healthy cobras were fed, each with 25 infected cyclops. The infected cyclops were poured down the throats of the snakes through a small glass funnel, more water being subsequently added to ensure that the cyclops passed through and that none of them remained sticking to the side of the funnel.

As a control experiment three healthy cobras were similarly fed each on 25 cyclops infected with embryos of the *human* guinea-worm.

All of these cobras died within the subsequent 12 months, but only two showed any abnormal post-mortem appearances :

- (1) This cobra died on the 16th May 1916, *i.e.*, two and a half months after being fed upon cyclops infected

\* In this connection it may be pointed out that the measurements given in the following two instances are obviously incorrect : Manson in his 'Tropical Diseases' 6th Edition 1916, p. 772, states 'The larva of *H. mediasiensis* measures about 15 to 25 mm. in length by 0·50 to 0·75 mm. at its greatest breadth.' Annett Dutton and Elliot in their 'Report of the Malaria Expedition to Nigeria,' Part II, Filariasis, p. 11, state : 'The embryos, . . . . . measure 15 to 25  $\mu$  long by 0·50 to 0·70  $\mu$  wide.'



FIG. 3.





with embryos of the cobra guinea-worm. There were no abnormal external appearances but upon dissecting the animal a worm about 3 cm. long and 1 mm. in thickness was found subcutaneously just under the abdominal scales midway between the head and the anal aperture. The worm was flat, transversely striated and showed contractile and relaxing movements. When it contracted it became shorter, thicker and broader; on relaxation it again became elongated thin and narrow. In this manner by alternately relaxing and contracting it progressed forwards.

- (2) The second cobra died on 3rd January, 1917, ten months after artificial feeding; this cobra, however, had been fed upon cyclops infected with embryos from a human guinea-worm. On dissecting this snake five worms were found within it. They were all flat, transversely striated and also showed alternate relaxing and contractile movements. Four of these worms were small, about 2 cm. in length and situated in the peritoneal cavity; they lay upon the intestines, and were partially embedded in the fat. One worm 4 cm. long, however, was situated just underneath the skin. They were exactly similar to those found in the other cobra.

I have not been able to recognize these worms, and as so little is known about the development of *Dracunculus medinensis* within the body of its definitive host, it is not possible to say that these represent undeveloped forms of a guinea-worm.

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SOME NOTES ON THE ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE IN MESOPOTAMIA, AND ON THEIR RELATION TO DISEASE.

PART I.

THE GAD FLIES OF MESOPOTAMIA.

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INTRODUCTION.

THE following notes, on some of the more important arthropods of medical and veterinary importance, were made when the author was serving with the late Mesopotamia Expeditionary Force, from November 1915 to August 1917. A large amount of material was collected at the time, but the publication of these notes was postponed, until all the material could be examined at leisure ; they are now published with the hope that they may be of some use to those medical and veterinary officers at present serving in that country.

The bulk of Mesopotamia is composed of rich alluvial deposits, forming a plain of great fertility, intersected by two rivers, the Euphrates and the Tigris ; the former, the longer of the two, flows through a depression, a few miles wide, and contains many islands, while the latter though shorter, contains far more water. The main branch of the

Euphrates joins the Tigris at Kurnah, but another, of which little is known, joins the Shatt-el-Arab at Gurmat Ali; the two rivers then flow to Basra as the Shatt-el-Arab, which enters the sea at Fao. The Karun flows in a tortuous course through the plains of Arabistan and enters the Shatt-el-Arab at Muhammerah.

The climate of Mesopotamia is subtropical and semi-arid with a somewhat small rainfall during the winter. June, July and August are the hottest months when the temperature may rise to 120°F and over, January is the coldest month, and in 1916 there was a fall of snow in the interior, a somewhat rare occurrence. The climate, on the whole, is preferable to that of Northern India. The cold season is a prolonged one and though the heat, during the day in the hot season, is intense and very trying, it is always possible to sleep at night without a punkah. Mesopotamia is near the south-eastern limit of the Palearctic Region, and in the sub-division known as the Mediterranean sub-region, and much of the fauna is European, particularly the birds, though many of the insects belong to the Oriental and Ethiopian Regions. As would be expected the riverine fauna is richer than that found in the desert areas, though the fauna of the latter contains a host of peculiar groups of insects, and many of the Diptera are predaceous and parasitic.

#### ORDER DIPTERA-BRACHYCERA.

##### *Family Tabanidae or Gad Flies.*

It might be thought that Mesopotamia would be rich in species of this family. The riverine belts of vegetation with their many creeks and watercourses, and the extensive marshy areas would appear to be ideal breeding grounds for these flies; but on the contrary only eight species belonging to three genera were found, and only two of these in large numbers. The explanation of this, however, may be due to the lack of sufficient cover, and the scarcity of suitable animals to feed on, and this suggestion receives some support from the fact that only two specimens of a dark species of *Hematopota* were taken. It is well known that these species of this genus, on the whole, prefer a somewhat dense scrub which is certainly absent in Mesopotamia.

##### *Genus Tabanus L.*

*Tabanus pulchellus* Lw. (*T. cyprinus* Ric.). (Plate LXVII, Fig. 1.)

A small light grey species, with yellow legs and clear wings; frontal



*Tabanus pulchellus* Lw., ♀ × 5.

*Tabanus glaber* Bigo., ♀ × 5.

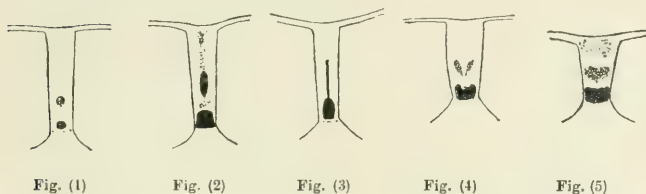




stripe broad, with two small round calli; abdomen with four somewhat indistinct spots, sometimes appearing as stripes, on each segment.

**FEMALE.**—Face and frontal stripe light grey, calli small, round, not reaching the eyes, lower just above angles of eyes, and upper about

TEXT FIG. 1.



Frontal stripes and calli of :—1. *pulchellus*; 2. *glaber*; 3. *polygonus*;  
4. *persis*; 5. *sufis*.

the middle of the stripe. (Text Fig. 1(1).) Antennæ yellowish, the third joint with a tinge of orange, and with hardly any tooth. Eyes dark brown, without any bands. Thorax greyish, with a suggestion of three dark bands. Abdomen yellowish grey, each segment, except the last, with four small, somewhat indistinct, dark dots or stripes on the fore borders. Legs yellowish with a tinge of orange; fore tibiae and all the tarsi brown to dark brown. Wings clear with a long appendix to the upper branch of the third longitudinal vein.

**MALE.** Usually smaller than the female, with the characteristic pointed abdomen. Upper large faceted area of eyes of the same colour as eyes of the female, lower small faceted area dark brown forming a striking band. Abdomen with first two segments reddish yellow at sides, and a dark stripe on each side of the middle line continued down the remaining segments.

This species closely resembles the widely distributed Oriental and Ethiopian *T. ditaniatus* Macq., and Major Auster, D. S. O., who kindly identified it, informs me it is probably only a local race of *ditaniatus*. It is recorded by Miss Ricardo from Kelopside, Cyprus, under the name *cyprianus*, from Seistan and from Persia where it is said to be a common horse-fly; it also occurs in Algeria. It is widely distributed in Mesopotamia, and is, together with the next species, one of the most important insect pests. It appears early in April in small numbers, but later in

the season is extremely common, only disappearing in September and early October. In the marshy areas along the Euphrates it literally swarms, and bites from early morning till dusk. At one camp, where the author was stationed, on the edge of a marsh, it was present in thousands during the months from May to July. A channel was cut across the shallow marsh and was marked by large stakes driven into the mud in order to guide boats bringing food to the camp. The water in this area was on an average of from 2 to  $2\frac{1}{2}$  feet deep, and the top of the stakes projected about a foot above the water level. The females selected these stakes on which to lay their eggs, and it was a remarkable sight to see one literally covered with egg masses, so that not a square inch was left bare; such a stake, on a rough computation, must have contained a thousand or more egg masses.

The mules and horses in this camp suffered severely from the effects of the bites of hundreds of these flies, and as a result became anæmic and thin. Various deterrents were tried but with little success, and the only certain means of protection afforded, was by housing the animals in reed huts. Very few flies entered these huts, and those which did were immediately killed. The writer was frequently bitten on the legs and arms when going about the camp. The females travelled long distances from their breeding grounds into the desert and were caught on many occasions from five to six miles away. Were it not for a large species of Bee-eater (*Merops apiastor* ?) which fed on the flies, and the myriads of large dragon flies, which also destroyed them, it would have been impossible to have kept animals in the camp.

*Tabanus glaber* Bigot (Plate LXVII, Fig. 2). A small dark reddish species with black legs and clear wings. Frontal stripe somewhat narrow, with three distinct calli; abdomen with a dark central stripe.

FEMALE.—Frontal stripe and face grey; lower callus square, almost black, and reaching the margins of the eyes, above it an elongated callus spindle-shaped, but sometimes bluntly shortened, often joining above a broad irregularly shaped callus on the summit of the vertex. (Text Fig. 1(2).) Thorax black with grey tomentum. Abdomen, first segment with orange spots on lower and outer sides, which are more evident on the second segment; remaining segments with slight indications of orange spots, or stripes, on their lower margins; under side reddish. Legs black, knees of fore tibiæ and whole of mid and hind tibiæ orange. Wings clear, without an appendix to the upper branch of the third longitudinal vein. Male unknown.

This species is recorded from Afghanistan, Kashgar, Eastern Turkestan, Seistan and Persia; it is widely distributed in Mesopotamia. It was very common during the end of the season from about August to November. Large numbers were caught on dromedaries in a camp in the neighbourhood of Nasiriyah, and these animals were at the time suffering from trypanosomiasis; all eventually died or had to be shot. This species was breeding along the banks of a creek in the vicinity of the camp; it does not appear to breed in marshy areas.

*Tabanus polygonus* Walker. A large reddish brown species with light central triangular spots on the abdomen; wings clear; frontal stripe with a broad square callus at the base, and a narrow black stripe extending as a rule from its upper border but sometimes free.

FEMALE.—Face grey, frontal stripe yellowish grey with a large brown quadrangular-shaped basal callus, narrowest at the base, and widest in the centre, not touching the eyes, middle callus forming a brown stripe invariably extending upwards from the basal callus. (Text Fig. 1 (3).) Antennæ reddish with the apical segments dark brown, almost black. Eyes dark greenish, without any visible band. Thorax dark with golden tomentum, with indications of a central light stripe and two lateral ones. Abdomen reddish with greyish triangular spots and dark markings at the sides, a characteristic central dark band on the upper two-thirds of the first segment. Legs red, all tarsi dark, more particularly those of fore legs. Wings without an appendix.

MALE.—Very similar to the female. Eyes with the large faceted area of a light brown colour, small faceted area of same colour as eyes of female, forming a dark band. Thorax darker than in female. Abdomen almost wholly red with the same black stripe on the second segment and the triangular grey spots smaller than in the female.

This species was first described from Baghdad, but it was found to be common all over the country. It could always be caught on horses and mules from March to September. It mainly breeds along the banks of creeks.

*Tabanus pulverifer* Walker. A rather small species with grey spots on the abdomen. It comes very near the European *Tabanus cordiger* Wiedemann, but differs from it according to Miss Ricardo in the following respects:—Antennæ wholly red, black at the apex. Forehead one-third narrower anteriorly, about four times as long as broad, frontal callus reddish brown. Coxæ and femora reddish yellow, as are the tibiæ, only the anterior tibiæ with a brown apex; tarsi reddish brown.

all with white pubescence. The under side of the abdomen has no median black stripe. The species is much lighter in colour and slighter in form than *cordiger*.'

Only one specimen of this species was taken in the marshy area along the Euphrates not far from Kurnah: it is recorded from Baghdad and from Turkey in Asia.

*Tabanus persis* Ricardo. A small species, superficially resembling *pulchellus*, with yellow legs and clear wings without an appendix; abdomen greyish with light central and lateral stripes; frontal stripe broad with two well marked broad calli.

FEMALE.—Face and frontal stripe grey, basal callus black, large, almost square, and nearly reaching the eyes, usually irregular along its upper border, the median callus variable in shape, often broken up, and sometimes heart-shaped. (Text Fig. 1(4).) Antennæ wholly black which helps to distinguish it from *pulchellus*. Eyes brown, without any evident band. Thorax greyish, with a suggestion of two short central and two long lateral dark stripes. Abdomen greyish, with a somewhat broad light grey central stripe on the first segment, narrower on the second, and faintly marked on the remainder. All segments with lateral spots or stripes directed obliquely from the centre. Legs yellow, apex of fore tibiæ and tarsi black, middle tarsi with last four joints dark, posterior tibiæ almost entirely brown. Wings clear.

MALE.—Very similar to the female, the abdomen reddish with whitish yellow pubescence.

This species is recorded by Miss Ricardo from Afghanistan, Seistan and Persia, and is said to be common on horses and camels. In Mesopotamia it was almost always found in company with *pulchellus*, but never in large numbers. It breeds in the same places, and it seems very probable that the small numbers, as compared to those of *pulchellus*, may be due to the larvæ being outnumbered by those of that species.

*Tabanus safis* Jannicke. (*T. albocentralis* Newstead.) A small blackish grey species, with yellowish legs, and clear wings; frontal stripe very broad with divergent sides, with a large black basal callus and two round central ones placed side by side. Abdomen blackish grey, with a median dark line and diffuse spots.

FEMALE.—Face grey, frontal stripe very broad, sides diverging towards the vertex, and about twice as long as broad, with a large black quadrilateral basal callus touching the eyes, and a middle callus consisting sometimes of two distinct circular dark spots. (Text Fig. 1(5).)

Antennæ brown, third joint without any tooth or projection. Eyes banded, ground colour yellowish green, upper band narrow and brownish in colour, second and third bluish green, and lowest band dark brown. Thorax brown, with four distinct white bands, the middle pair extending beyond the transverse suture. Abdomen blackish grey with a faint central grey band, and dark lines extending from the upper borders of the segments obliquely to the lower borders of each segment, most marked on the second segment, outside these, diffuse grey spots obliquely directed. Legs yellowish, all femora brown, to dark brown, two-thirds of front tibiæ yellowish grey, remainder, together with tarsi, brown, mid and hind tibiæ yellow except for dark band at tip, mid and hind tarsi yellowish, with apical segment dark. Wings clear, with a long appendix to the upper branch of the third longitudinal vein.

MALE.—Very similar to the female, eyes with upper large faceted area dark grey, and lower small faceted area with coloured bands, as in female. Thoracic markings not so distinct as in female. Abdomen redder than in female.

This species is recorded by Surcouf from Jerusalem, Abyssinia, the Anglo-Egyptian Soudan, Mauretania (Morocco and Western Algeria), Senegal, and from Gambia by Newstead under the name *alboventralis*. It is found in many parts of Mesopotamia, though it is not a common species. It breeds along the banks of creeks, and along water courses.

*Genus Chrysops* Meigen.

*Chrysops punctifera* Lw. A small greyish yellow species, with golden pubescence. Antennæ mostly dark brown. Thorax with two dark lateral bands. Abdomen yellowish, second segment with a V-shaped spot. Legs yellowish. Wings white, costal border brown, with one clear oval spot occupying almost the whole of the discal cell.

FEMALE.—Face greyish yellow, frontal stripe broad with a well-marked black heart-shaped basal callus. Antennæ with basal segment wholly orange with some dark apical hairs, remaining segments dark brown. Eyes with the usual bands characteristic of the species of the genus. Abdomen yellow, first segment with two dark spots coalescing at base of segment and diverging, but not reaching lower border, second segment yellowish at base, with a V-shaped mark not reaching upper border of segment, third and remaining segments with dark basal bands with slight indications of V-shaped spots. Legs yellowish, all femoro-tibial joints black, most marked on front legs, front tibiæ yellow, lower

third dark brown, all front tarsi dark brown, mid and hind tibiae yellow, and lower segments of tarsi brown. Wings white, with whole of costal margin light brown and with a broad brown band extending across the wing from about the middle, nearly up to the fork of the third longitudinal vein, an oval white spot occupying almost the whole of the discal cell.

MALE.—Almost wholly black; antennæ entirely black. Thorax black, with a central broad grey band just extending beyond the transverse suture. Abdomen black, first segment entirely black, second with two light yellow spots at sides and a small spot at centre of lower margin, third segment with a narrow band extending more than half way across the border, fourth segment with a similar but longer band. All legs entirely black. Wings darker than in female, discal spot much smaller.

This species was common at Basra along the creeks and water-courses during the months of April and May; it was also present along the Euphrates and Tigris.

#### *Genus Hamatopota* Meigen.

Only two specimens, and these males, were taken during the whole time the author was in the country; as they are now headless it is not possible to describe them. The species of this genus are evidently rare in Mesopotamia.

### GAD FLIES AND THE TRYPANOSOMIASIS OF DROMEDARIES IN MESOPOTAMIA.

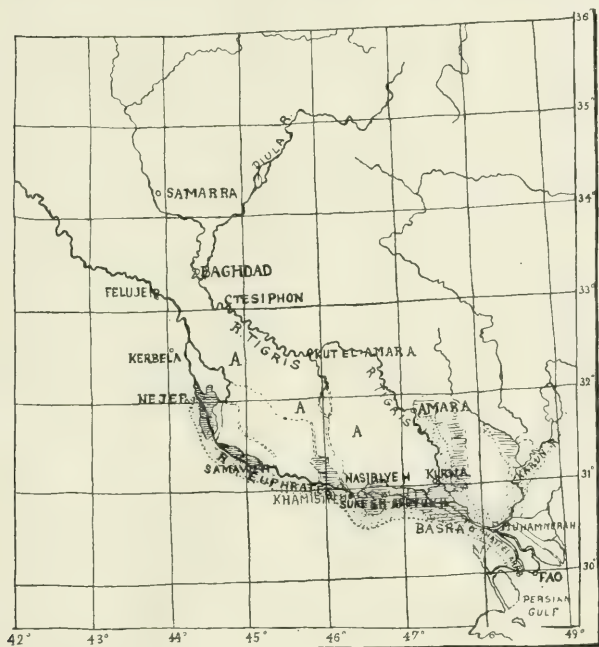
During the late campaign more than a thousand dromedaries belonging to the various camel corps, then in the country, were lost as a result of a very fatal form of trypanosomiasis. Each dromedary is worth about Rs. 70, so that this represented a very considerable loss, quite apart from the crippling of the corps as far as their utility was concerned. The disease was widespread occurring along the Karun, Euphrates and Tigris.

The dromedary, as is well known, is one of the animals most highly valued by the Arab, and particularly the desert nomad whose very existence depends on it. There are two types of dromedary in the country, the riding variety which is highly bred, of light build and capable of great and continuous exertion, and the baggage variety of



heavy build, capable of carrying from 300 to 600 lb., if properly loaded. The desert Arab values his dromedaries as his life, and well he may; the milk of these animals is highly nutritious, the flesh makes excellent food, and the hair warm clothing. The nomad Arabs who live in the desert areas, bordering the rivers, particularly the Euphrates, breed

TEXT FIG. 2.



Map of Lower Mesopotamia shewing Gad Fly Belts (dotted). A. The area between the Euphrates and Tigris said by the Arabs to be free from trypanosomiasis. Marshes (.....)

these animals in large numbers, and many of the influential Sheiks own a thousand, or more head. One of the Sheiks living some distance inland from Khamasiyah told the writer that he owned 1,000 dromedaries and that though the annual mortality among his animals varied from 8 to 10 per cent., none of them died from trypanosomiasis. He evidently knew



the disease very well, describing the symptoms accurately, and associated it with the bite of the gad fly—a specimen was shewn him which he called *zareji* (an Arab name, probably common to all biting flies). One per cent. of his dromedaries were over 9 years old, 5 per cent. over 8, and the remainder under 6 years. The animals over 10, and up to 14 years were always killed and eaten. He ascribed the freedom of his animals from this very fatal disease to : (1) the knowledge that the disease was associated with the bite of the gad fly, and (2) to the knowledge that there is a fly belt, ten miles in width, extending along the right bank of the Euphrates from Fao to Nejif and possibly further north. (Text Fig. 2.) His motto was 'Prevention is better than cure,' and though he professed to cure the disease, the author was not impressed with the results. The dromedary, like the other members of the family, cannot do without water, and his animals were brought to drink daily, some five miles beyond the head of a creek where there was a water hole. They were driven there at dusk when the flies had stopped biting and immediately after being watered, were taken back to his camp which was well beyond the fly belt. He recognised the importance of not exposing his animals to the bites of gad flies, and thus he said he never lost a single animal from trypanosomiasis. The dromedaries belonging to the various camel corps, however, were, for obvious reasons, kept well within this fly belt, in fact only a few hundred yards from the edge of the river, and as a result, they all became infected and died. Among these animals there were a few Sind dromedaries and none of these, as far as the author knows, became infected, though some of them had been there during two fly seasons ; this seems to suggest that they were immune to the Mesopotamian variety of trypanosome.

In one camp, situated close to the river, just above Nasiriyah, a number of animals recently purchased from an Arab Sheik were collected during the months of July, August, September and October ; they had not been there very long before they began to sicken, and soon shewed large numbers of trypanosomes in their blood. The writer visited this camp on many occasions, and it was here that *T. glaber* was found in large numbers feeding on the dromedaries ; except for a few specimens of *T. pulchellus* it was the only species seen ; and there were no other biting flies, but large numbers of a new species of hematophagous *Musca* which will be described in another paper. It then occurred to the author that this outbreak afforded a good opportunity of investigating this problem. For here were ideal conditions, and with only one species of

*Tabanus* to deal with. A large number of specimens of *glaber* were collected and dissected and it was found that 100 per cent. were infected with a species of *Crithidia* closely simulating *C. tabani* Patton; a large percentage contained the characteristic postflagellate stages massed in the hind gut, and the contents of the rectum contained large numbers of flagellates. No true trypanosomes were found in the flies, and there were no parasites in the salivary glands. Although the number of glands examined was small, the negative results suggested that the trypanosome did not undergo any developmental changes in the flies.

Knowing from previous studies on the *Crithidia* parasitic in gad flies, that they are acquired by the adult flies in the act of sucking blood, it was only necessary on this occasion to watch these flies feeding, to see that this was most probably the only way by which they became infected. *Tabanus glaber*, and indeed most gad flies which attack dromedaries, bite them on the belly, chiefly in the neighbourhood of the genital organs, udder, and penis sheath, and inside the thighs, a few on the neck and sides, and some on the legs. As the flies feed, they eject a dark fluid from their alimentary tracts, and sometimes pure blood, on to the skin and hair of the animal which can be seen as drops sticking either to the skin or hair. This fluid on examination proved to contain large numbers of flagellates and the postflagellate stage referred to above. Before sucking blood these flies search for a suitable spot applying their labella to the hair, and then the skin, and in this way they ingest the *Crithidia*. The dromedaries were noticed to be driving the flies away by licking the sites where they were biting, and it was clear that at each act they were infecting their lips with the various stages of the parasite. It therefore occurred to the author that this trypanosome in the blood of the dromedaries may be nothing else but a stage of this *Crithidia* parasitic in the fly, and that when it came in contact with the mucous membranes of the mouth, it penetrated it, entered the blood and there assumed the trypanosome stage. There is nothing startling in this hypothesis, for it is well known that trypanosomes can penetrate the skin and certainly the mucous membrane; it has been proved that many species of the genera *Herpetomonas* and *Crithidia* can find their way into the blood by way of the alimentary tract. This has been repeatedly demonstrated by Laveran and Franchini, and many others, in the case of several of these purely insectan parasites.

The writer attempted to carry out this experiment, but under very imperfect field service conditions. Two young dromedaries, which were

at the time free from trypanosomes, were isolated for two months in a camp known to be free from flies on the left bank of the Euphrates at Nasiriyah ; at the end of this time they were still free from trypanosomes. A large number of specimens of *glaber* were collected from the camp mentioned above, and dissected, and the various stages of the *Cerithidia*, only from the hind guts, were placed on the mucous membranes of the mouths of the animals. Unfortunately the experiment could not be carried to its conclusion as the writer was moved to another part of the front, and the animals were lost sight of. This experiment should be carried out under more favourable conditions, and the results recorded ; it would at least dispose of one possible way in which gad flies may infect dromedaries with trypanosomes.

THE TRYPANOSOMIASIS OF DROMEDARIES IN OTHER  
PARTS OF THE WORLD, AND OUR PRESENT  
KNOWLEDGE OF THE METHOD OF TRANS-  
MISSION OF THE CAUSATIVE AGENTS.

Trypanosomiasis of dromedaries is a disease which is prevalent in many parts of the world, and more particularly in Africa and India. *Mbori* is the name given to one form of it which occurs among dromedaries in the Sahara and the Soudan ; Cazalbou has made a very complete study of it. It is common in the valley of the Middle Niger, and in the neighbourhood of Timbuctoo and on the west of Lake Faquibin is said to be endemic and causes very considerable losses. Laveran and Mesnil state that it is carried by the caravans conveying rock salt from the mines of Taoudine to Timbuctoo, from the mines of Tichit to the country of the Upper Senegal, Northern Nigeria, and Lower Senegal ; also from the oasis of Bilma to the country round Lake Chad and to the region of Zinder. In the Anglo-Egyptian Soudan the same disease is common among the local dromedaries, and causes great mortality ; it is also reported from the Transvaal by Theiler, and from Italian Somaliland, where it is locally known as *Salef*, by Martaglio. The Arabs from the Sahara believe that this disease, *Mbori*, results from the bites of gad flies, and Cazalbou records the ubiquitous *T. ditaniatus* Macq., *T. rufipes* Macq., and *T. gratus* Lw., from the area. The trypanosome of *Mbori* is believed to be a variety of *T. evansi*.

Another form of trypanosomiasis occurring along the whole of the African Mediterranean littoral, and particularly in Algeria is known locally as *El Debab* (*debab* being the Arabic name for a gad fly). The

brothers Sergeant have for many years investigated this disease. It is also believed to occur in Egypt, but it is not certain whether the trypanosome (*T. soudanense*) is the same as that of the Algerian form. Dromedary owners in North Africa have long believed that the gad fly, so common in their country in the summer months, is the cause of the disease. These men know that when the dromedaries are kept in localities where gad flies are numerous, the mortality among their animals from *El Debab* is very high; but that in places where these flies are rare the disease is practically absent. The brothers Sergeant believe that *Tabanus (Atylotus) nemoralis* Meig. and *Tabanus (Atylotus) tomentosus* are the two species specially concerned in the transmission of the trypanosome of *El Debab*. These authors have carried out a long series of very careful feeding experiments with various local biting flies, and they found that the trypanosome is transmitted from one animal to another mechanically; that is to say the flies are perforce intermittent feeders, being driven off when they alight on an animal, they attack again, and so on, until they have fully fed. Such a fly commencing to feed on an infected animal is driven off before it is able to become replete with blood, and then may alight on a clean animal and infect it. The Sergeants found that infection was positive only after a short interval (15 to 70 minutes), and that a fly kept for a longer period, as a rule, did not infect a clean animal when applied to it. One experiment was positive when a species of *Stomoxys* was used, but the majority failed. The trypanosomes were destroyed in less than one hour after entering the stomach of the gad fly.

In Egypt, *Tabanus teneola* Pallissot de Beauvois, a widely distributed species, and a smaller, much more localised one, are said by Laveran and Mesnil to be the carriers of the *T. soudanense* of the Egyptian form of *El Debab*. Bouet and Roubaud succeeded in transmitting the trypanosome of *Tahuga (El Debab?)* in the Soudan through the bites of *Stomoxys calcitrans* and *Stomoxys bouvieri*.

Turning now to India for our knowledge of the trypanosomiasis of dromedaries the writer is mainly indebted to the valuable work of Pease, Montgomery, Leese and many other Veterinary Officers. Pease has contributed a noteworthy article on *Tibarsa Surra*, a form of trypanosomiasis common among the dromedaries in the Punjab and North West Frontier Provinces. This disease, known under a variety of local names, has in the past caused a heavy mortality among the Silladar Camel Corps. Pease says that so far as his experience goes, 'all Indian camels

irrespective of breed and age are susceptible to the disease provided they are taken into the Surra zones during the fly season.' In India too the dromedary owners associate the disease with the bite of the gad fly.

Montgomery draws attention to the serious mortality from the disease among the Silladar Camel Corps, and mentions that over 5,000 camels died from trypanosomiasis during the late Seistan Boundary Commission.

A large number of transmission experiments have been carried out by Leese with various biting flies in the Surra zone at Mohand on the Saharanpur-Dehra-Dun road at the foot of the Siwalik Range. This author found that the local gad flies (*Tabanus* and *Hematopoda*) readily infected healthy animals acting as mechanical agents; the time of transfer from the infected animal to the healthy one was from half to three minutes. Experiments with *Stomoxys* were on the whole negative, though some positive results are recorded. Mosquitoes and sand flies failed to infect when transferred from infected to healthy animals.

From this short and somewhat incomplete summary of the observations made on the trypanosomiasis of dromedaries in various parts of the world, it will be seen that the evidence regarding the method of its spread by gad flies is very conflicting, and they do not afford a satisfactory explanation of the method of transmission of the trypanosomes concerned by these flies. Our present knowledge appears to be that gad flies only act as mechanical carriers, and that the trypanosomes soon disappear from their alimentary tracts, and that only those parasites which chance to be on the labella and prestomum are carried to healthy animals. If this is the case it is difficult to understand why the gad fly is the only certain carrier, when it is known that many other species of biting flies are just as intermittent in their feeding habits, for example the many species of *Stomoxys* and the hematophagous species of the genus *Musca* which are always present in the company of biting flies all the world over. It should be noted also that all feeding and transmission experiments with gad flies have been carried out with *wild* specimens and not with flies *bred* in the laboratory.

In order to settle this vexed question the writer would suggest the following line of investigation:

(1) A careful survey of all the species of gad flies which bite dromedaries in a selected trypanosomiasis infected zone; their correct identification, seasonal prevalence, biting and breeding habits.

(2) A careful and complete study of the flagellates of the genera *Crithidia* and *Herpetomonas* which may be parasitic in any of the species, quite apart from any flagellates the flies may ingest from the blood of their hosts.

(3) Transmission experiments with any or all of these purely insectan parasites:—

- (a) by application of the parasites to the unbroken skin,
- (b) by application to the broken skin,
- (c) by application to the mucous membrane of the mouth,
- (d) by subcutaneous inoculation of dromedaries proved to be clean, and in a locality other than the trypanosome infected zone.

(4) Feeding experiments with *bred* gad flies on animals infected with a known strain, and the study of the evolution, if any, undergone by the trypanosomes in the organs of the flies.

Unfortunately, in the case of gad flies, such a complete series of investigations would be hard to carry out owing to the difficulty in rearing the early stages to maturity as most of their larvæ are predaceous; they are not easily found in nature in sufficiently large numbers. But they can be bred out by keeping each larva in a separate receptacle. Transmission experiments could be conducted in large wire enclosures containing suitable animals and breeding places.

#### THE CONTROL OF GAD FLIES IN MESOPOTAMIA.

It will be noted that dromedary owners in Mesopotamia avoid the fly areas, and are well acquainted with the belts along the rivers where the gad flies abound, particularly in the summer months. Their animals are kept outside these belts, and are only brought into them to be watered, and then only at dusk when the flies are not on the wing; and always driven away before the flies appear in the morning. If forced to graze their animals in the infected areas they do so only in the early morning and late afternoon, when only stray specimens of gad flies are on the wing. As the breeding grounds of gad flies cover enormous areas of country in Mesopotamia it would be quite impossible to attempt to destroy the early stages. Much might, however, be done to destroy the eggs by introducing into the country those species of Chalcids which are parasitic on the eggs of the flies: the writer never saw any infected egg masses. Cavalry officers would do well to remember that though the writer only saw one horse which was infected with



trypanosomes, this disease may quite well spread to these animals with disastrous results. All horses should be protected from these flies by housing them in reed huts as deterrents are of little or no use.

In conclusion the author hopes that these notes may rouse some further interest in this disease of dromedaries, and that the investigations suggested will be carried out, either in Mesopotamia or in India. He understands that an enquiry into the method of transmission of the trypanosomes of dromedaries is now being undertaken in the Anglo-Egyptian Soudan. He will be glad to give any further advice regarding the gad flies of Mesopotamia, and to identify any specimens sent to him. The next paper will deal with the Mesopotamian house flies and their allies.

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SOME NOTES ON THE ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE IN MESOPOTAMIA, AND ON THEIR RELATION TO DISEASE.

PART II.

MESOPOTAMIAN HOUSE FLIES AND THEIR ALLIES.

BY

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ILLUSTRATED

BY

EDITH M. PATTON.

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IN a previous paper the writer described the gad flies of Mesopotamia, pointing out that the two species, *Tabanus pulchellus* Lw., and *T. glaber* Lw., are associated with the transmission of the trypanosome found in the blood of the dromedary of the country. It was suggested that this trypanosome may only be a stage in the life history of the species of *Crithidia* parasitic in the alimentary tracts of the two species mentioned above. Our present knowledge of the method of transmission of the trypanosome pathogenic to dromedaries, through the agency of gad flies, was summarised, and it was pointed out that the usually accepted explanation that these flies only act as mechanical carriers was unsatisfactory, in view of the fact that other biting and non-biting flies were just as intermittent in their feeding habits as the gad flies. A line of investigation which

was needed to solve this problem was outlined. The possibility of this disease affecting horses in Mesopotamia should be borne in mind, and all measures should be taken to protect these animals from the bites of these flies; and above all dromedaries should not be kept in the riverine fly belts, which are danger zones and recognised as such by the Arabs.

In the present paper the various house flies and their allies, which are of economic importance, will be described, and the best measures for controlling these insects given in some detail.

ORDER DIPTERA : SUBORDER CYCLORRHAPHA.

*Family Muscidae : Sub-family Muscinae : Genus Musca\* :*

*The House Flies.*

Mesopotamia, in addition to being renowned as the birth place of the human race, also enjoys the unenviable reputation of being the home of the house fly. Those who were in the country during the late campaign will recall their experiences of a plague of flies, which enabled them to appreciate what Pharoah and the ancient Egyptians had to contend with when the land of Egypt was plagued with these insects. The extraordinary rapidity with which they appeared in myriads in a camp, and especially in the vicinity of towns and villages, was little short of miraculous, and under such conditions life was intolerable. To see hundreds of flies crawling over one's food is bad enough, but to experience the sensations produced when they settle in large numbers all over the body is far worse: rest during the day becomes impossible unless under a mosquito curtain, and relief only comes at night, when the last meal can be taken in comfort.

*Musca determinata* Walker. A typical member of the genus, with four black thoracic stripes, and closely resembling the European species, *Musca domestica*, but differing in some small, but definite details, which will be fully described in another paper.

Male. Front black, narrow about  $\frac{1}{2}$  width of head; palpi and antennae dark. Thorax ground colour slate grey, with four moderately broad black bands, the median pair, in many specimens, appearing to

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\* For many years the writer has collected and studied a number of species of the genus *Musca*, and hopes soon to contribute a series of comparative studies with a view later to publish a revision of the genus. There is at present the utmost confusion regarding even the commonest species, and the field worker and sanitarian have no means of identifying their specimens. In order to make these studies as complete as possible, the writer will be glad to receive species of the genus from any part of the world.



PLATE LXVIII.



*Musca determinata* Walker, ♀, X 8.



*Musca humilis* Wied., ♀, X 8.

blend with the outer pair. Scutellum grey with a dark apex and, in many specimens in addition, a dark band down the centre. Abdomen, first segment black except at the lower border and sides which are orange; in dark specimens the whole segment is black. Second segment light to dark orange, central black band broad and T-shaped with the limbs of the T extending to a varying extent along the upper border of the segment. This T-shaped band is very constant and was present in many hundreds of specimens examined. There is also a shimmering grey patch on each side of the central black band, and a similar patch on the outer edge of the segment; the intervening area is usually dark orange, but in dark specimens forms a tawny band or spot. The grey bands are well marked in dark specimens, but indistinct in light ones. Third segment similar to the second, the central band is narrower and the grey and dark bands more pronounced. Fourth segment dark grey in the centre with well marked lateral dark bands, and grey patches at the sides. Legs black; wings hyaline and venation typical of the genus.

Female. (Plate LXVIII, Fig. 1.) Front broad about width of the head. Thorax and scutellum similar to those of the male. Abdomen darker, with shimmering greyish yellow patches and dark bands giving it a chequered appearance; a black median band as in male, but narrower. First segment dark orange to black with a broad black central band; in the majority of specimens the markings are similar to those of the male, except that there is often a black band extending along the lower border of the segment. Second segment ground colour grey with a central black band, shimmering grey patches on each side and edges of the segment, and a dark band between the grey bands. Third segment similar to second central band narrower and lateral dark bands between grey patches often appear as isolated spots. Fourth segment similar to that of the male, grey patches duller.

Holding a female sideways with the light falling on the abdomen and examining it with the naked eye, the lateral dark bands stand out as dark patches, triangular in shape, with the base of the triangle resting on the lower border of the segment. These patches together with the shimmering grey ones and the dark central band give the whole abdomen the chequered appearance noted above. The lateral dark bands are evidently the tawny spots referred to in Walker's description.

This species was described in 1856 by Walker from the West Indies, his description, however, would apply equally well to the typical *Musca domestica*. It has been recorded from various localities in India, and is

probably widely distributed; it is the common house fly of Bombay, but has not so far been found in South India. It is common throughout Mesopotamia, Aden, and the Aden Hinterland. Recently the author collected many specimens in the bazaar at Port Said; it is common in Egypt and probably throughout Palestine.

*The early stages of Musca determinata.* The egg of *determinata* is exactly similar to that of *domestica*; it measures about  $1/25$  of an inch in length. The larva, when full grown and ready to pupate, is creamy white in colour and measures about  $\frac{1}{2}$  an inch in length. The anterior spiracles consist of 7 processes and the posterior spiracles of the mature larva are depicted in Text Fig. 2. The puparium is of the usual *Musca* type and is of a dark mahogany colour.

*Life history and breeding habits.* The eggs are laid in batches of from 150—250 or more, and oviposition is completed in two to four such batches. During the summer months in Mesopotamia, the eggs hatch out in from 6 to 8 hours. In June eggs laid in the morning had hatched by the afternoon, and by the following evening were near maturity. The larval stage then lasts from 36 to 42 hours, and the adults emerge 36 hours after pupation; under such conditions, and with a rich food supply one generation succeeds another in from 72 to 90 hours. In the Mesopotamian cold weather all the stages are retarded. The egg stage may last as long as 24 hours, the larval stage 72 hours or more, and the adults hatch out in from 72 to 96 hours after pupation.

As a result of a long series of breeding experiments carried out in the field by placing the various larval food stuffs in suitable receptacles close together, it was found that the female *determinata* oviposits in the following, and in the order given below:—

1. Human excrement, and the green partly digested food from the stomachs and intestines of cattle and sheep; these are preferred to any other by the female fly as food for the larvæ. It was noted that the larvæ, bred in the latter, matured more rapidly, and attained their maximum size, and that the adults hatching out of such larvæ were large and vigorous. This fact is of considerable interest and shows that the house fly is essentially a vegetable feeder in its early stages, and confirms the conclusion reached by Newstead as the result of his well-known Liverpool experiments. On two occasions many thousands of eggs were seen laid at random on the surface of a mass of the green stuff referred to above, making it appear as if it had been dusted with sugar through a sifter. On the first occasion this phenomenon was noted,

the green food contained a seething mass of larvæ close to the surface, and the many female flies which came to oviposit, and began to insert their ovipositors into the material, were disturbed by the larvæ, and as a result laid their eggs on the surface. On the second occasion a similar appearance was seen on the surface of a large collection of partly disorganised human excreta from the middens of the Arab houses in Nasiriyah. These pits were cleaned out periodically, and the black masses were deposited just outside the town in a field. The men who did this work also poured water from the drain pits in the courtyards on to the black masses. As soon as the surface dried, flies began to lay their eggs in it, and owing to the enormous number of larvæ which were present, the females coming later deposited their eggs on the surface.

2. Horse and mule droppings. This food comes next in importance, and in the cold weather is the chief breeding material for the fly; in the hot weather it dries too rapidly, and unless in large heaps is unsuitable for the larvæ. In the cold weather, however, relatively small collections remain moist and there is sufficient warmth for the development of the larvæ.

3. Decaying vegetables, tea leaves, and other kitchen refuse come next at suitable breeding grounds.

4. Cowdung. This is utilised to a small extent, and, as far as the writer was able to ascertain, only in the cold weather and when collected in large heaps. Patches of cowdung dropped in the field were never used by *determinata* in which to oviposit.

As is well known, the larvæ pass down into the food mass and remain hidden from view, but during the night come up to the surface, and when they have eaten through a mass of horse dung, it presents a characteristic appearance. The heap which was probably irregular at the surface is now flattened out and presents a worm eaten appearance due to the larvæ having passed through it over and over again. This is a point of practical importance, for it enables the observer to detect a breeding ground with certainty, and when once noted it can be readily recognised again. When the larva is mature there is a short resting period during which time the alimentary tract is emptied, and it now assumes its characteristic creamy yellow appearance due to the large collection of fat body which forms a continuous sheet under the skin. The larvæ are now ready to migrate, and they do so usually at night, leaving the mass from the base, and rapidly crawling away to select a suitable *nidus* in which to pupate; sometimes however they pass down into the earth directly



below the mass, provided it is moist and there are cracks into which they can worm themselves. The extraordinary power the larva has of burrowing into the earth, even when hard, is remarkable. Pupation as a rule never takes place in the foodstuff. The adults, on hatching, nearly always collect together in the vicinity of the breeding grounds when copulation takes place; they then scatter to their feeding grounds. This is a point of practical importance, for if the observer finds a small swarm of house flies collected together in any locality, and more particularly if they fly on to any moving objects, and settle in a clump, they are almost certainly recently hatched flies and the breeding ground will be near by. On many occasions the writer was able to locate a breeding ground by noting this phenomenon.

*Musca humilis* Wied. (*Musca angustifrons* Thomson; *Musca cuteniala* Walker; *Musca conducens* Walker; *Musca prator* Walker.) A small to medium-sized greyish species with two black thoracic bands and a chequered abdomen.

Male. Front narrow about  $\frac{1}{2}$  width of head; palpi and antennae black; frontalia and cheeks silvery white. Thorax ground colour yellowish grey with two very broad black stripes reaching the posterior margin, and enclosing a yellowish grey stripe, narrow at the anterior end and widening out posteriorly. Scutellum greenish to yellowish grey. Abdomen orange with silvery reflections; first segment entirely black; second orange, with a broad black central band and silvery reflections next to band and on outer sides; third segment orange with a narrow central black band, silvery reflections and a dark band often marked along lower border; fourth segment dark grey with patches of black at the sides. Wings hyaline.

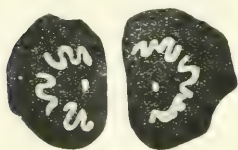
Female. (Plate LXVIII, Fig. 2.) Front yellowish grey, broad about width of head; palpi and antennae black; frontalia and cheeks silvery white. Thorax yellowish grey with two black bands, each formed by the union of the two black bands the outer of which is separated from the inner at the anterior end, but joins it at the transverse suture. Scutellum grey and dark in the centre. Abdomen greenish grey with silvery reflections giving it the chequered appearance noted above; first segment black as in the male; second segment with a central dark stripe, adjacent silvery patches, and brown at the sides; third segment similar except that the central stripe is narrower; fourth segment dark grey with silvery and brown reflections, and dark patches on each side of the middle line.

*Musca humilis* is a widely distributed species, probably even more so than the type species of the genus (*Musca domestica*). It is found along the North African coast, is a common bazaar fly in Egypt and the Soudan, in Port Said, Aden and the Aden Hinterland, and in many parts of India where it may be seen in large numbers in the bazaars sitting on foodstuffs and particularly on raw meat; it occurs throughout Mesopotamia and probably in Palestine. It is also found in many parts of China and in Australia. It occupies an interesting position in the genus for it is a connecting link between the house fly group and the hæmatophagous species. It may be seen on horses and cattle feeding on sores and cuts, and on the blood which exudes from the bites of true biting flies.

This species was described by Thompson from China under the name *angustifrons*, but Stein has recently pointed out, that it is identical with Wiedemann's species *humilis*, which name antedates *angustifrons*. Recently while in England the writer took the opportunity of examining specimens of *Musca euteniata* Bigot, in the collection at the Natural History Museum, and came to the conclusion that it is identical with *humilis*. Comparison of *humilis* with Walker's species *conducens* leaves no doubt in the author's mind that they are identical, and Walker's short description of this species supports this view. It is very probable that *Musca præcox* Walker is another synonym for *humilis*. It is interesting to note that *euteniata* has been recorded by Smith from Benares as being a troublesome house fly breeding in cowdung cakes. For long the writer could not understand this peculiar breeding habit, for no other species, frequenting houses in India, is known to the author to breed in cowdung cakes. Now that the identity of *euteniata* is cleared up, the peculiar breeding habit is readily explained, for *humilis* regularly breeds in cowdung, and especially in patches dropped in the field.

*The early stages of Musca humilis.* The egg of *humilis* is so similar to that of *determinata* that they can hardly be distinguished, but if anything it is smaller. The larva is also like that of *determinata* being of the same creamy white colour. The anterior spiracles usually consist of six processes. The posterior spiracles, however, differ very considerably from those of *determinata*. In the case of *humilis* (Text Fig. 1) each plate is of the usual D-shape, black throughout, the outer margin often appearing irregular, and the three spiracular slits show up as light wavy lines. The stigmal plates of *determinata* (Text Fig. 2) on the other hand are rounded, the whole of the outer margin is of

a light brown colour, and the three slits appear to run into each other, and form a reddish crescentic-shaped mass. These differences can be readily seen with a hand lens magnifying about 10 to 15 diameters.



TEXT FIG. 1.



TEXT FIG. 2.

TEXT FIG. 1. Posterior spiracles of *Musca humilis*.

TEXT FIG. 2. Posterior spiracles of *Musca determinata*.

The puparium of *humilis* is very similar in colour to that of *determinata*, so that it is almost impossible without examining the posterior stigmata, to distinguish one from the other; the differences between the stigmal plates of the two species noted above should, however, be looked for.

*Life history and breeding habits.* The eggs of *humilis* are laid in somewhat smaller batches than in the case of *determinata*, otherwise their life histories are very much the same. The larvæ are capable of developing to maturity in a number of food stuffs, and may be found in patches of cowdung dropped in the field in heaps of horse manure, in human excreta, either when in large quantities, or in a single dropping, and in the green partially digested food from the alimentary tracts of sheep and cattle. It is important to note that the species breeds in patches of cowdung, and in isolated patches of human excreta.

#### MESOPOTAMIAN HOUSE FLIES AND DISEASE.

A visitor to any of the towns or villages of the country cannot help being struck by the appalling amount of ophthalmia prevalent among the inhabitants. Every second person met in the streets has one or more of the sequelæ of this disease, and the cause is not far to seek. It is only necessary to go into one of the court yards where the children are kept, to see a small baby in a rude cradle with its eyes covered with *Musca determinata* and *Musca humilis* (the latter species is extremely fond of sucking up fluid from sores), to understand how the infection is

carried from one child to another. Sore eyes are neglected, with the result that blepharitis, corneal ulcers, etc., soon follow and the sight becomes permanently impaired.

The organisms of infantile diarrhœa, enteric fevers, dysentery and cholera are unquestionably carried by these flies, and the sanitary officer should therefore be thoroughly acquainted with their breeding habits. During the late campaign those units which neglected to prevent the breeding of house flies in the vicinity of their camps, suffered from small epidemics of these preventable diseases. The writer recalls one such case, in which the sanitation of the camp was left to look after itself, and flies were allowed to breed in large numbers in the conservancy area ; refuse of all kinds, empty tins, etc., were thrown outside tents, and no attempt was made to protect food from flies, with the result that there was a heavy sick rate from paratyphoid fever and diarrhœa among the men of the unit. This case left no doubt as to the important part house flies play in the mechanical transmission of disease-producing bacteria. In a country like Mesopotamia, where the climatic conditions are eminently suitable to the rapid production of house flies, good sanitation is of the utmost importance, and the European population can only hope to keep fit by paying particular attention to the control of these injurious insects.

#### THE CONTROL OF HOUSE FLIES IN MESOPOTAMIA.

In order to control the house fly, and for the matter of that any injurious insect, it is first necessary to study its life history, in order to discover the weak points, which can be selected for attack. It is not only important to know the species, but also everything that can be learnt about it, where, and under what conditions it lays its eggs, and the habits of the larvæ and adults. Fortunately there are many weak points in the life history of the house fly. First it should be noted that it is a gregarious insect, lays its eggs together in masses in foodstuffs suitable for its larvæ, and the latter on hatching out keep together, and later migrate from the breeding ground in order to pupate. They can only reach maturity when the temperature, moisture, and fermentation are at their optimum. An increase of one of these factors reacts on the other and upsets the balance ; for instance, an increase in fermentation leads to an increase in temperature and liberation of gases which kill the larvæ. The mature insects collect together after hatching when copulation nearly always takes place ; this preoviposition period constitutes a fifth stage

in the life history of the fly. After copulation and before the eggs are ripe both sexes scatter from their breeding grounds, and are attracted by offal and blood, and swarm around butcheries and slaughter houses.

The writer will now describe, how, after studying the life histories of the two house and camp flies of Mesopotamia, the prevention of breeding and the destruction of flies was accomplished at a fixed camp at Nasiriyah, observations which later led to the successful control of these insects throughout the occupied parts of the country.

The camp at Nasiriyah was situated on both banks of the Euphrates, and at various times accommodated from 10,000 to 20,000 troops; that on the left bank extended down to the town which had a population of about 10,000. A central conservancy depôt was established at a suitable site on each bank, and here large open grid incinerators were built to burn equine manure, food refuse, offal, etc.: the space between the incinerators was utilized for drying the manure. A small area was allotted to Hindus and another to Musalmans where they could slaughter their animals, the rule being that the abdominal viscera had to be opened up, and all the undigested food and any offal had to be placed on a layer of dried manure, thoroughly mixed into it and allowed to dry for a few hours after which it was placed on the incinerators.

Each unit had its own sanitary area with a closed incinerator, and every day dried equine manure, sufficient to burn the excreta from the unit, was issued in gunny bags from the central depôt, and the sweepers of the unit mixed this with the excreta from the latrines and burnt it in their own incinerator. All food refuse, including tins, etc., was collected in carts from all the conservancy areas and taken to the central depôts where it was dealt with by the sweepers of the Sanitary Section. All equine droppings were sent daily by each unit to the central depôt, and each unit deputed one or more men according to the number of bags they sent, to help to deal with it at the central depôt. The men working at the depôts were under the orders of a sergeant of the Sanitary Section, and one or more British soldiers. From 600 to 800 bags of equine droppings were brought daily to the central depôts; the offal and contents from the A.S.C. slaughter house were sent by that unit to the depôts.

At each depôt a small shed covered with mats was erected close to the incinerators, and was used, as will be described later, to destroy adult flies. It will be seen then that house fly control at this fixed camp was based on the prevention of breeding: the larval foodstuffs being destroyed before the flies had time to lay their eggs, and any that were laid and the

larvæ which had hatched were burnt. In all fixed camps, therefore, it is important to burn all human excreta, all equine droppings and especially all the contents of the viscera of slaughtered animals; Indians if not watched will bury the latter, with the result that a large number of flies are bred out of it. Closed incinerators are to be preferred to open ones, for incineration of the various larval food stuffs in open incinerators always allows a certain proportion of larvæ to breed in them, and this is generally due to the difficulty experienced in getting the large masses to burn uniformly, many parts remain unburnt sufficiently long to allow eggs to hatch and larvæ to become mature, and then they crawl over the sides or pass into cracks and pupate in the ashes. Flies hatching out from these situations often have particles of ash clinging to their bodies, especially the thorax, and flies shewing this appearance indicate that the incinerator is not working efficiently. All cracks should be filled up, and the incinerator should never be overcharged, but the mass of manure should be below the level of the walls; pupæ should be swept up and placed in the fire. In working an open grid incinerator, the men should be instructed to see that the excreta from the latrine is thoroughly mixed with dry equine manure, and when placed on the incinerator this should be covered with a dry layer several inches deep; flies do not care to oviposit in dry horse manure. If these precautions are taken the number of larvæ breeding in open incinerators may, for practical purposes, be ignored.

During the hot weather, horse manure is best spread out in a uniform layer about 4 inches deep, turned over once or twice during the day and then collected in a heap for distribution, or for immediate burning on the incinerators. The drying ground should be hard and free from cracks so that no manure can collect in them. In the cold weather in Mesopotamia a different method can be adopted, especially when there is a large amount of manure to be dealt with; if it is spread out to dry, it is apt to remain moist, owing to heavy dews, for a sufficient time to permit of breeding. To obviate this the writer experimented with the biological method of dealing with manure, which as Roubaud has pointed out, is used to breed, and at the same time destroy house fly larvæ.

Roubaud, in describing the biothermic method of treating fresh manure, points out that the droppings from a single horse in France are sufficient to give rise to from 40,000 to 50,000 house flies during the summer. In June as many as 160,000 to 200,000 may be produced. Oviposition takes place in the stable before the droppings have been



removed, and this occurs regularly in November and December in Mesopotamia. Oviposition continues up to 24 hours, but after that time excessive fermentation sets in and protects the manure from the laying of eggs, and this is an important point to note. All antiseptic substances such as borax, cresol, ferrous and ferric salts, etc., when applied to the manure retard fermentation and prolong the period for deposition of eggs, one or two days longer. In the same way when used as larvicides they prolong the period of infestation, and produce the very opposite result to that aimed at. From the sixth day, manure, to which fresh droppings have not been added, when placed in a heap contains no larvæ, for they have either migrated away from the heap or have burrowed in the earth below it. Anti-fly measures should therefore be taken within five days of the removal of the manure from the stable, on the second and third day in the tropics. The eggs which are in the heap, having been deposited in the manure before collection, hatch out and the larvæ make their way to the superficial layers of the heap in order to escape the heat developed as a result of fermentation in the central parts. On the following day a temperature of from 158° F to 194° F may be recorded in the centre of the manure heap; and such a heap can be utilized to destroy the larvæ. The larva of the house fly, if protected from the gases of fermentation, dies in three minutes when exposed to a temperature of 112° F, and when exposed to the gases it dies in a minute at a temperature of 123° F; in 5 to 7 seconds at 138.2° F and 4 to 5 seconds at 140° F. When such a manure heap, containing larvæ in its superficial layers, is turned over, they are killed when they come in contact with the hot parts from the interior. A complete stirring up of the heap, to which no fresh manure has been added, on four consecutive days causes a disappearance of 90 per cent of larvæ. Roubaud suggests that the destruction of the eggs and larvæ is more easily and quickly accomplished if, instead of waiting until the infected heap has produced the necessary temperature, it is exposed to the heat and gases of a heap previously fermented. Instead of placing the fresh manure on the surface of the heap as is usually done, it should be buried in the hot parts of the fermented heap, and covered with a layer of hot manure just over four feet thick. In about five hours the fresh manure may be considered to be free from eggs and larvæ which would otherwise have developed in thousands; further it should be noted that flies cannot reach it in order to oviposit. This biological method, or delarvization by heat, is equivalent to the heating of the entire fresh manure to a temperature of from 112° F to 145° F,



and is accomplished without any apparatus or fuel. In practice it is found that the mass of fermented manure required to produce the necessary temperature is about eight times that of the fresh manure to be treated. The next day this buried manure is used as a source of heat. Covering fresh manure with earth, straw, etc., or saturating it with chemicals does not prevent the development of the eggs already deposited in it, and chemicals retard fermentation thus prolonging the period of deposition and infestation.

During the winter of 1916-17 the writer gave the biothermic method of dealing with equine manure a thorough trial, to see how this method would work in a tropical climate. Large quantities of fresh manure were heaped into mounds varying from 20 to 25 feet in height, and 30 to 40 feet in circumference with flat tops. Each mound was placed on a selected piece of hard ground, and consisted of two days' collection of fresh manure. On the third day the manure from the centre of mound No. 1 was removed, and the fresh manure of the day buried in the hole, and covered with the hot manure; and this was done in the case of each of the other mounds. By the time the last mound was thus utilised, No. 1, and part of several others, had been removed and burnt in the incinerators, and fresh mounds started. In this way the fresh manure was each day exposed to the heat and gases in the mounds, and all eggs and any larvæ were destroyed. But it was soon found that both *determinata* and *humilis* began to breed on the layer of manure on the outer surfaces of the sides and tops of the mounds. The manure in these situations, though exposed to the heat of the sun during the day, remained moist, owing to the heavy dews common at this time of the year, and the larvæ were able to obtain sufficient moisture to enable them to reach maturity; they could not, however, pass into the mound for more than 4 to 5 inches on account of the great heat. This then constituted a fresh problem, and one not mentioned by Roubaud as occurring in France, and had to be dealt with immediately, as owing to the enormous surface available for breeding many millions of flies would have been produced. It was noted that the larvæ on migrating from the mound crawled, but mostly rolled, down the sides of the mound, and left it at the circumference; and here was a weak point in the life history of the insects especially suitable for attack. A small shallow trench 6 inches wide and 4 inches deep, with straight steep sides, was constructed to encircle each mound, and it was at once found that the larvæ on trying to leave the mound fell into the trench and had perforce to pupate

in it as they could not scale the sides. But in order to encourage them to remain in the trench, and not to burrow into the ground—for it is astonishing how deep a muscid larva can burrow even into ground as hard as a rock,—a thin layer of dried manure was spread along the bottom of the trench. Each morning one or more men swept up the manure, containing larvæ and puparia, with small brooms, and placed them in empty kerosine oil tins, and the day's collection was buried in the hole of a hot fermenting mound when all were very soon destroyed. In addition it was found that large numbers of immature larvæ could be collected by brushing off the outer layer of manure to a depth of 4 inches and dealing with it in a similar way. As a result of these experiments the writer can thoroughly recommend the biothermic method of dealing with large quantities of equine manure particularly in tropical countries, bearing in mind that the larvæ may breed on the surface of the heap.

One more instance of how large numbers of larvæ may be dealt with will be given. At one time or another the writer found enormous numbers of larvæ, especially in the huge masses of the green partially digested food from the viscera of slaughtered animals at the slaughtering ground of the town of Nasiriyah. The question arose as to how these larvæ could be destroyed in these semi-liquid masses. The writer knew that it was only necessary to dig a trench six feet deep, four feet wide and about 10 feet or more long, and to leave it for a night, and next morning it always contained from two to four feet of water, depending on the locality. The mass of green stuff with the contained larvæ, and with all the puparia which should be collected was thrown into the trench and all were drowned. This is another instance of a weak point in the life history of the house fly which can be utilised for attack, for it is well known that the larvæ and puparia of these insects are readily drowned. On another occasion in a camp the writer was living in, it was noticed that *Musca determinata* and *M. humilis* suddenly appeared in large numbers, and for a time he was at a loss to locate the breeding ground. After considerable search, it was found that the flies were breeding in decaying fish which had become stranded in a channel about  $\frac{3}{4}$  mile from the camp on the windward side. During the flood season this channel had filled with water, and as the river fell it slowly emptied itself until it again became dry. Thousands of young fish were caught in this trap and could not escape: they were heaped in masses in some places 1½ feet deep. As putrefaction set in *Musca*

*determinata*, *M. humilis*, *Chrysomyia albiceps*, *Lucilia sericata*, and *Calliphora erythrocephala* laid their eggs in the dead fish, and soon the bodies became a seething mass of larvæ. How were these larvæ to be disposed of? Burning was out of the question. A deep trench was dug and as soon as water percolated into it to a depth of 3 feet, the dead fish and contained larvæ, as well as all the puparia which could be collected, were thrown into the trench, which was left open long enough to ensure that all were drowned. The nuisance was thus quickly brought under control. The Sanitary Officer in Mesopotamia will do well to bear in mind this stranding and decaying of young fish, and to remember what an excellent breeding ground the dead bodies become for the house fly and its allies.

All the methods utilised for attacking the larval stages of the house fly having now been described, it remains to mention the methods used for dealing with the adult insects.

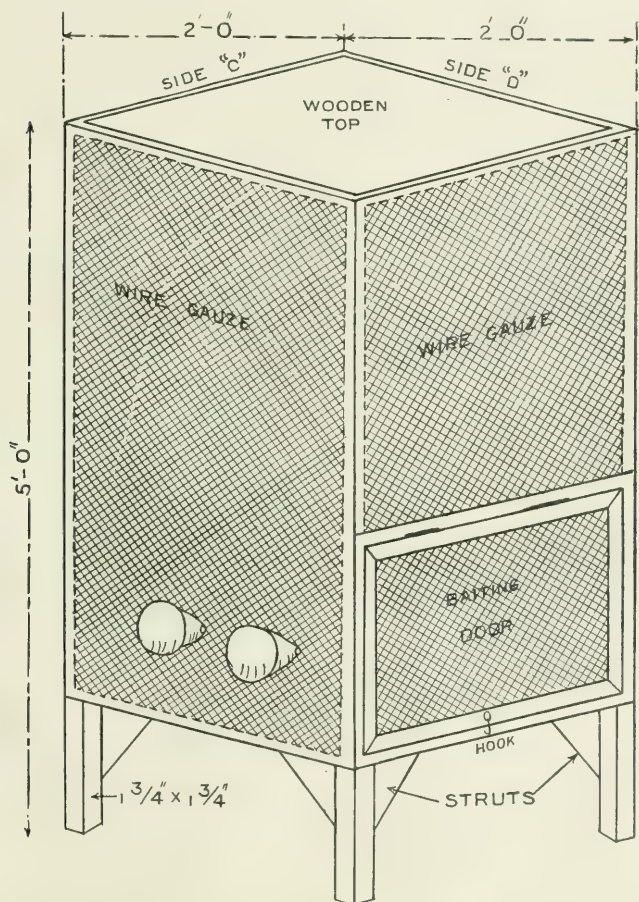
It will be remembered that at each central depôt a small shed was erected consisting of four mud walls 3 feet high with a door, and the whole covered with matting supported on four posts buried in the walls, and leaving the sides open for about 3 to 4 feet. This shed was a collecting place for flies, both *determinata* and *humilis*, which had just hatched out from larvæ which had escaped destruction, also for females which had come to oviposit. In the evening the inside of the roof and the sides were literally black with the insects. Just at dusk when they had settled down for the night, and were unable to fly far, five men were provided with kerosine oil torches made from rags collected at the depôts, four were posted at the sides and the fifth entered the shed. The torches on being lighted were rapidly applied to the masses of flies, and any which attempted to leave the shed were caught by the torches at the sides. In a very short time all the flies were destroyed, most, however, only having their wings singed, and next morning the dead flies, and those which were only able to crawl, were swept up and placed in tins and burnt in the incinerators: it is no exaggeration to say that often half a tinful of dead flies were collected. The matting was never set on fire and it was surprising to see how efficient the men became after a very short time.

Where a unit has a small open incinerator it is a good plan to erect a roof of mats over it, sufficiently high to prevent the smoke interfering with the flies. In the winter months house flies collect in large numbers on the under surface of the roof which gives them shelter and warmth and

then can be burnt in the way described above. This method of dealing with the adult insects is a good example of the importance of observing every little point in the life history of an insect; and the knowledge that the house fly, in its preoviposition stage, has the habit of collecting in the neighbourhood of its breeding grounds can be utilised to great advantage in destroying these insects, before they scatter to their feeding ground where they chiefly infect food with bacteria. On one occasion at a camp on the edge of a marsh *determinata* and *humilis* became a veritable plague, owing to the fact that the Commandant of the camp was entrenching all human excreta in shallow trenches, and the writer was called upon to proceed to the camp, and deal with the nuisance. The mischief was already done and there was very little hope of collecting the larvæ and puparia. Entrenching was at once stopped and incinerators erected, but the flies continued to hatch out in myriads and caused great discomfort. It was noted that on going to the old entrenching ground a swarm of flies got up from it, and alighted on one's person, chiefly the back and helmet, so that every one who went to the place brought back hundreds of recently hatched flies to the camp. From his previous experience of dealing with this stage of the house fly, the writer at once erected a large shed consisting of four long posts, the roof and the two sides in the direction of the wind being covered with mats, and the other two sides left open. Each evening at dusk, men with torches burnt thousands of flies which collected in black masses on the insides of the matting. In a short time the camp was cleared of flies.

The last method of dealing with the adult insects in camps is by using properly constructed and baited traps. The principles adopted in the construction of the many traps which have been described, is to bait them with some food which is especially attractive to the adults, which enter the trap through an opening so arranged as to permit of easy ingress, but difficult exit. In some traps the method of entry on the contrary seems to be particularly difficult, and in others again the entrance to the trap also serves as an excellent exit, allowing the flies to pass out after having had a meal. Many kinds of mixtures have been concocted, all of which aim at only attracting the insects to enter the trap to feed. The writer has long adopted the principle that the bait used in the trap should not only be a food for the flies, but also a medium especially attractive to the gravid females which is the most important sex to trap. After

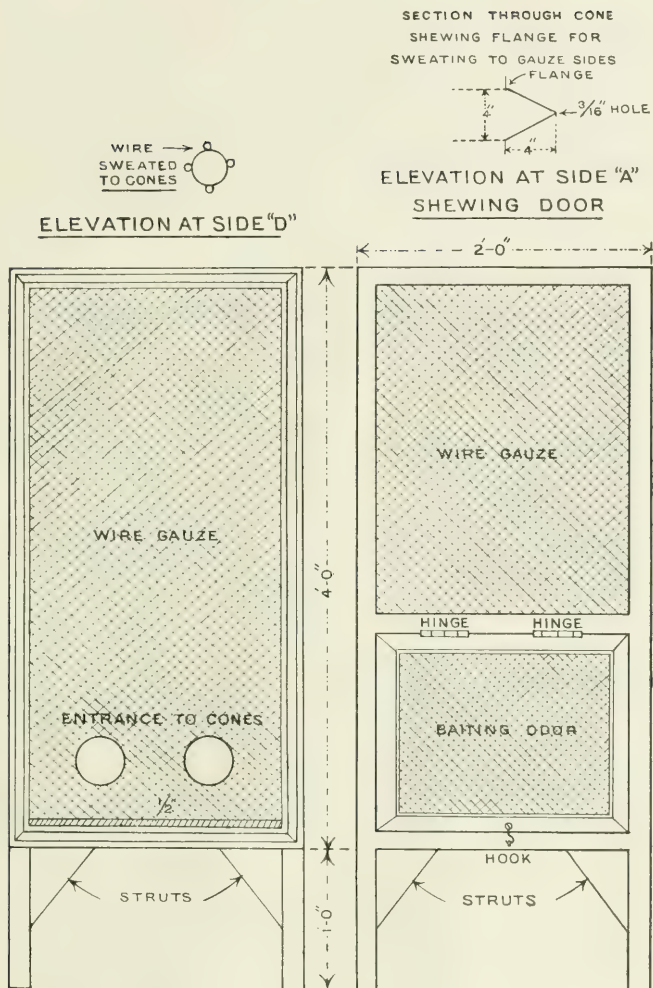
experimenting along these lines the writer found that the green partially digested contents of the stomachs and intestines of recently slaughtered cattle is *par excellence* the food most attractive to females about to



TEXT FIG. 3.—Large fly trap devised and used by the author in Mesopotamia.



oviposit, and especially so when a few clots of blood are placed on the surface. This material is placed in a specially constructed trap which will now be described. The trap (Text Figs. 3 and 4) as will be seen from the drawing, consists of a large meat safe on legs. It is 5 feet in height, one of which is taken up by the legs: the sides are 2 feet wide and are covered with wire gauze, the top and bottom being made of wood. The door for baiting the trap is situated at the lower end of one side, and opens from below upwards, and the bait is placed in a tin tray on the floor. The entrance to the trap consists of 6 gauze cones, 4 inches in diameter at the entrance, and projecting 4 inches into the trap, narrowing down to an opening  $\frac{3}{16}$  " in diameter: there are no cones on the door. The cones are so placed as to open almost at a level with the bait, and this is a point of considerable importance, for it was found after placing the cones at various heights, that most flies entered by those placed on a level with the bait. The explanation seems to be that the smell emanating from the bait is best detected on a level with it. The method of constructing the trap is made clear in the drawings, and any intelligent carpenter, given the materials, can make one. The only difficulty lies in sweating the cones on to the wire gauze so as to make the entrances flush with it. The trap is baited in the early morning, by piling up the food on the tray and placing clots of fresh blood on the surface, and is left until dusk, when the tray is removed and the contents, which contain large numbers of eggs, and even larvæ are placed in an incinerator. The great advantage of this trap is that it requires little or no attention when once baited, and there is no need to kill the flies by spraying them, etc., as they can be left to die. It will be found that the females, after laying their eggs, soon die and fall to the bottom, and it is only necessary once or twice a week to brush out the dead flies. At night it will be noted that those which are still alive have collected on the under side of the roof; and most of these will be dead next day. No difficulty is experienced in baiting the trap, as the door opening from below upwards does not permit of the escape of the trapped flies. One of these traps was placed close to the conservancy area of a Field Ambulance, and was worked by an Indian sweeper in a most efficient manner. The writer can confidently recommend the trap to the Sanitary Authorities in Mesopotamia, and there is no reason why it should not be used in Indian Cantonments, for the bait is always available at a slaughter house.



TEXT FIG. 4.—Fly trap shewing side elevations and other details in its construction.



*Musca mesopotamiensis*, sp. nov. A comparatively large hæmatophagous species, superficially resembling *Musca pattoni*, but readily distinguished from it by the width of the front in both sexes; the differences will be fully described in another paper.

Male. (Plate LXIX, Fig. 1.) Front narrow about  $\frac{1}{3}$  width of head. Antennæ dark grey, palpi black. Frontalia and cheeks silvery white. Thorax ground colour bluish grey, with four black longitudinal stripes extending the whole length of the dorsum; scutellum the same colour as the thorax with a suggestion of a central dark area. Abdomen orange with silvery reflections; first segment orange with a central dark band varying in width in different specimens, in some the band widens under the scutellum; second segment orange with a broad central band, sometimes with the lower margin of the first segment black giving the appearance of the letter T, dark patches at sides and margin of segments with silvery reflections; third segment dark orange with a narrow central band, darker areas at sides and silvery reflections at margins; fourth segment dark orange, varying in depth of colour in different specimens, with an indistinct dark central band, and sides with silvery reflections.

Female. (Plate LXIX, Fig. 2.) Front wide about  $\frac{1}{3}$  width of head. Antennæ and palpi as in male. Frontalia and cheeks silvery white. Thorax lighter than that of the male; and scutellum of the same colour as the thorax. Abdomen greenish grey with a tessellated appearance; first segment dark orange to black, with silvery reflections best seen in fresh specimens; second segment grey with two lateral triangular-shaped dark spots, the apices directed towards the first segment, and silvery reflections on the outer sides of the spots; third segment grey with a central, often triangular-shaped, dark band, dark spots at the sides similar to those on the second segment, except that as a rule their apices do not reach the second segment; fourth segment grey, with a central dark area.

Early stages of *Musca mesopotamiensis*. The egg of *Musca mesopotamiensis* is very similar to that of *Musca domestica* but is much larger; unlike that of the other hæmatophagous species it is not armed with a spine. The larva when mature is of a creamy white colour, and measures about  $\frac{5}{8}$ ths of an inch. The anterior spiracles are broad and have from ten to twelve processes. The posterior spiracles consist of large black plates markedly kidney shaped. The puparium is of a dirty white colour, very similar, in general appearance, to that of *Musca pattoni*, but can be readily distinguished from it by the large black

# PLATE LXIX.



*Musca mesopotamiensis* Sp. nov., ♂, X 8.



*Musca mesopotamiensis* Sp. nov., ♀, X 8.



770<sup>b</sup>

PLATE LXX.



*Musca tempestiva* Fallen., ♀. X 10.



*Musca vitripennis* Meigen., ♀. X 10.

stigmal plates, as compared to the much smaller plates of the larva of *pattoni*.

*Life history and breeding habits.* The eggs of this species, some 30 in number, are always laid singly in patches of cowdung dropped in the field. The larvæ, when ready to pupate, migrate from the patch, and bury themselves deeply in the ground at some distance from it; some, however, may be found below the patch of dung. A large number of puparia were collected during the end of October and in November and were kept in some earth, the flies from these puparia did not hatch out until the following March. The flies disappear in November and only reappear during the early days of March; no larvæ could be found in patches of cowdung during the cold weather, but on the other hand it was possible to find puparia in the ground in the vicinity of old patches. From these observations it is clear that this species hibernates during the winter in the pupal stage.

As already mentioned in Part I of these notes, large numbers of this species were seen on the camels referred to, feeding on the exudation from sores, eyes, etc., also on the blood coming from the bites of the true biting flies. Both sexes may be seen on cattle, horses, mules and donkeys flying from one animal to another, and worrying biting flies to try and induce them to withdraw their proboscides before becoming replete with blood; in this way the species may be a vector of disease-producing organisms. The flies are never seen in tents, houses or bazaars.

*Musca tempestiva* Fallen. A small dark grey species with four black thoracic bands.

Male. Front black, very narrow so that the eyes almost meet. Palpi and antennæ dark. Frontalia and cheeks silvery grey. Thorax and scutellum black. Abdomen grey; first segment entirely black; second and third segments grey, with a black central stripe, narrower in the third; fourth segment grey. Wings hyaline, bend of fourth vein not acute.

Female. (Plate LXX, Fig. 1.) Front wide about  $\frac{1}{3}$  width of head. Antennæ and palpi as in male. Thorax dark grey with four somewhat narrow black bands, the middle pair ending just below the transverse suture. Scutellum grey. Abdomen dark grey; first segment black throughout; second segment mostly black, with grey patches at the sides; third segment grey with a black central band; fourth segment dark grey with darker patches at the sides.

This small species was described by Fallen in 1820, and is widely distributed in Europe. It is common in Mesopotamia and can always be seen in the summer months on horses and cattle, following biting flies and behaving like *Musca mesopotamiensis*. Its early stages are not known, but as it was often seen sitting on patches of cow dung it is very probable it breeds in this substance.

*Musca vitripennis* Meigen. A small metallic species probably not belonging to *Musca* but to *Placemyia*; the eyes in the male are hairy.

Male. Front black, very narrow; eyes hairy and almost meeting. Frontalia and cheeks silvery. Thorax and scutellum dark green. Abdomen orange; first segment entirely black; second, third and fourth segments orange, with a broad black central band, and silvery reflections on sides. Wing hyaline, bend of fourth vein not acute.

Female. Front broad about  $\frac{1}{3}$  width of head. Palpi and antennæ dark. Frontalia and cheeks silvery. Thorax green, with four narrow longitudinal stripes, the middle pair hardly extending beyond the suture. Abdomen orange.

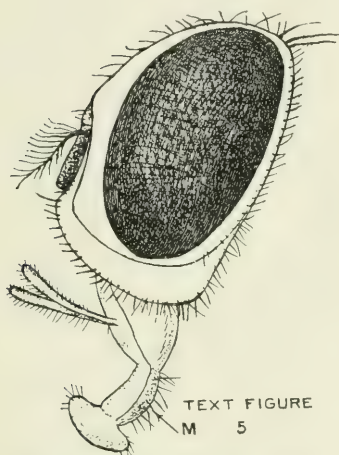
This species is widely distributed in Europe and North Africa. It is common throughout Mesopotamia, and like *tempesta* is seen on horses and cattle following the biting flies and sucking up any blood which exudes from their bites. Its early stages are not known, but it probably breeds in patches of cow dung dropped in the field. It is very probable that this species and *tempesta* occur in North India.

#### SUB-FAMILY PHILÆMATOMYIINÆ: GENUS PHILÆMATOMYIA.

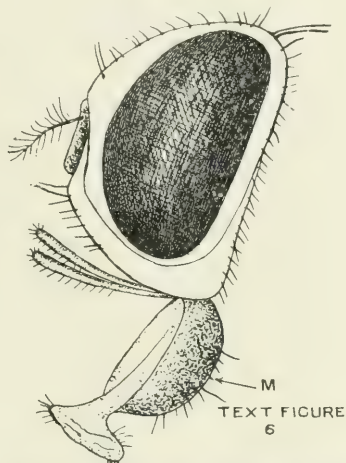
*Philæmatomyia crassirostris* Stein (*insignis* Austen) was the only species of the genus which was seen, and it was very plentiful on cattle all over the country. It is so well known, having been fully described and illustrated by the author in collaboration with Major Cragg, F.M.S., that it is not necessary to describe it again. Yet the author finds it is often confused for *Musca*. The points to note are that *Philæmatomyia crassirostris* is of a greyish yellow colour with light thoracic stripes especially in the female while the thorax in the male is much darker and the stripes are better marked; the female eyes are widely separated; the front being much wider than in most species of *Musca*; in the male the front is narrow. But the best way of distinguishing the species of the genus from *Musca* is by examining the proboscis, which should be pushed out either with the aid of a needle or by compressing the body of the fly. In *Philæmatomyia* the mentum is well marked, and is



seen as a dark boat-shaped mass of chitin, while in *Musca* the mentum is feebly developed and is seen as a delicate plate of chitin (see Text Figs. 5 and 6). Further, in a side view of the head in *Philematomyia* the posterior border of the eye is straight, but in *Musca* it is more or less convex. The teeth at the end of the proboscis of *Philematomyia* can often be seen projecting between the everted labellæ.



TEXT FIG. 5.—Side view of head of *Musca nebulosa* shewing proboscis extended; note the small mentum (M) as seen in a freshly killed specimen.



TEXT FIG. 6.—Side view of head of *Philematomyia crassirostris* shewing proboscis extended; note the large boat-shaped heavily chitinous mentum (M) as seen in a freshly killed specimen.

#### SUB-FAMILY STOMOXYDINÆ: GENUS STOMOXYS.

Only one species, *Stomoxys calcitrans*, of this genus was met with in Mesopotamia; it is so well known that a description is not necessary. It can be very easily recognised while biting horses and cattle by noting that it keeps its wings well apart, and the long proboscis cannot be missed.

This fly was a great pest at Nasiriyah during the flood season, large numbers coming into the tents during the day and freely attacking the occupants, biting the legs through stockings and very often through putties; it will also bite on bare arms though it seems to prefer inserting

its proboscis through some hairy substance. In the Hummar Lake they flew into the boats and were a great nuisance.

*Genus Lyperosia.*

*Lyperosia exigua.* One of the largest species of the genus, slate grey to dark grey in colour. Thorax ash grey to dark grey, with two narrow well separated stripes. Abdomen dark grey with a dark longitudinal median band on the first and second segments. Third and fourth veins converging towards their extremities, and first posterior cell narrowly open.

This species is widely distributed throughout Asia, India, and Mesopotamia. It is commonly seen sitting on the shoulders of cattle with its head directed downwards.

*Lyperosia minuta.* A small dark grey species. Thorax brown with two brown lateral stripes. Abdomen dark grey without any distinct markings.

This small species is found throughout India and in many parts of Africa ; it is very common in Mesopotamia.

FAMILY CALLIPHORIDÆ : SUB-FAMILY CALLIPHORINÆ :

GENERA CALLIPHORA, CHRYSOMYIA AND LUCILIA.

*Calliphora erythrocephala* Macq. The well known European Blue Bottle, or Blow Fly. A large bluish black species with a more or less whitish pollinose abdomen and black legs. Palpi reddish, antennæ black and jowls (part of face below cheeks) red, with black hairs. This species was very common during the summer months laying its eggs in meat, dead bodies of animals, offal and other animal refuse.

*Chrysomyia* (*Pycnosoma*) *albiceps* Wied. A medium sized bright metallic green species, some specimens dark green. Front narrow in the male, and wide in the female ; frontalia and cheeks, in male silvery with white hairs, in the female yellow with yellow hairs. Antennæ red buff coloured in female, in male first segment buff and remainder dark ; palpi buff coloured in both sexes.

This species is common all over the country and breeds in the same situations that *Calliphora* breeds in.

*Lucilia sericata* Meigen. The well known sheep blow fly. A medium sized bright metallic green species. Front narrow in male and wide in female. Frontalia and cheeks in both sexes silvery with dark

hairs on the jowls. This species can at once be separated from any other species of *Lucilia* by noting that the second abdominal segment has no marginal macrochètæ.

*Lucilia sericata* is a very widely distributed species of great economic importance as it has the habit of laying its eggs in the soiled wool of sheep, and causing considerable loss to farmers. It was never observed to do this in Mesopotamia, probably owing to the fact that it had much other animal matter always available in which to lay its eggs. In Scotland this fly regularly attacks sheep with soiled wool, whereas in the South of England the author collected many specimens but never saw or heard of a case of larvæ in sheep. Many specimens of larvæ were collected from wet mud contaminated with the urine of horses and the typical *sericata* was bred out of these larvæ. It could not be bred in the bodies of dead birds or small animals.

The Blow Flies can be readily controlled when we remember where they breed. All dead bodies of animals should, if possible, be burnt, and the same should be done with meat offal, etc. If the larval food stuffs are destroyed very few adults will be present. The bait used in the fly trap mentioned above, attracted many specimens of *Lucilia sericata* and *Chrysomya albiceps*, and the trap is very suitable for controlling these flies.

FAMILY SARCOPHAGIDÆ : SUB-FAMILY SARCOPHAGINÆ :

GENERA SARCOPHAGA AND WOHLFAHRTIA :

THE FLESH FLIES.

The flesh flies can always be recognised by their grey colour and often reddish eyes, striped thorax and chequered or spotted abdomens. The identification of species in the family is, however, extremely difficult, and nearly always necessitates making a caustic potash preparation of the external genitalia of the male.

Two important genera are found in Mesopotamia, viz., *Sarcophaga* and *Wohlfahrtia*, which can be separated by noting the following points.

*Sarcophaga*. The third segment of the antennæ is nearly twice as long as the second, the arista is armed with bristles almost to the apex. The abdomen usually has chequered markings.

*Wohlfahrtia*. The third joint of the antennæ is short, and the arista is not armed with bristles. The abdomen is marked with well defined spots and there are no glistening reflections as in *Sarcophaga*.

*Sarcophaga hæmorrhoidalis* Fallen. (Plate LXXI, Fig. 1.) A large typical species. Front of male narrow, that of female wider. Frontalia silvery. Antennæ and palpi brownish black. Thorax grey, pollinose, with three to five black stripes. Abdomen pollinose with changeable tessellation and three indistinct changeable stripes; last segment in male with a row of fourteen well marked bristles.

This species was very common during the hot weather, when the dromedaries affected with trypanosomiasis were dying in large numbers, and owing to the lack of fuel the bodies had to be buried, a difficult task in the case of such a large animal and *hæmorrhoidalis* found an excellent breeding ground for its larvæ. Females deposited their larvæ in the bodies long before decomposition set in.

The species is readily attracted to human excrement and breeds in it; it can be trapped by placing excrement in the tray as a bait. *Hæmorrhoidalis* has been recorded as causing intestinal myiasis, so that it may be well to know something about this species.

The writer collected large numbers of the larvæ of this species in the mud along the river banks near towns; the mud here contained a considerable quantity of animal refuse of all kinds, and it was evident that this species regularly bred in this situation. It may be well for Sanitary Officers to bear this in mind.

*Wohlfahrtia meigenii* Schiner. A large grey *sarcophaga*-like fly with characteristic round spots on the abdomen. Front narrow in male, wide in female. Frontalia silvery. Antennæ with arista plumose but not armed with bristles. Thorax with three well marked light stripes. Abdomen grey with round dark spots; first segment with three spots, one in the middle and one at each side, the spots extending almost the whole width of the segment; second segment with three smaller spots not extending to the upper margin of the segment, a median dark line extending from the upper end of the middle spot; third segment with similar though smaller spots; fourth segment with faint indications of spots.

This species is very common all over Mesopotamia, and deposits its larvæ in the dead bodies of animals. Other species of this genus have been recorded as attacking man and animals and depositing their larvæ in sores and even in the eye so that it is important to be able to recognise the larvæ. The larva of *Wohlfahrtia* may be distinguished from those of *Calliphora* and *Sarcophaga* by noting that the spines on the middle segment are stronger and scattered all over the segment without any definite

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*Sarcophaga haemorrhoidalis* Fallen., ♂, X 6.



*Wohlfahrtia Meigenii* Schiner., ♀, X 8.



order. In *Calliphora* the spines are much smaller, and are arranged in a line along the margins of the segment. In *Sarcophaga* on the other hand the segment appears to be bare, but if examined with a high power it will be seen to be covered with small pale coloured spines densely arranged on the surface of the segment. No case of myiasis caused by the larvæ of *Wohlfahrtia meigenii* was seen.

This concludes the notes on the more important Mesopotamian House Flies and their allies. The writer will be glad to identify any of these species and to give any further information which may lead to their more efficient control. The next paper will deal with the Bot Flies of Mesopotamia.



# THE USE OF HYDROCYANIC ACID GAS FOR FUMIGATION.

BY

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HYDROCYANIC ACID GAS has been used for many years for killing insects. The most familiar method of using this gas for this purpose is found in the naturalist's killing bottle. In this case the insect is first caught, it is then placed in the bottle and removed after a variable period when found to be dead.

According to Woglum, James T. Bell in 1877 seems to have been the first person to use hydrocyanic acid gas to kill insects which were not in the first instance caught. In Volume 9, pages 138-140, of the Canadian Entomologist, he described a method of killing insects with hydrocyanic acid. The insects in this case had invaded an entomologist's cabinet, and the gas was generated by dropping sulphuric acid upon potassium cyanide.

But it was not till 1886 that hydrocyanic acid gas was more extensively used for destroying insects. In that year hydrocyanic acid gas was used for killing the cotton cushion scale in orange orchards in California by Coquillett. Fumigation with this gas is now practised on a large scale in California, Florida, Australia, South Africa, Spain, Porto Rico and Japan, especially in connection with the fruit-growing industry. This gas has also been used for the fumigation of vermin-infested houses, ships and railway carriages in America, Australia, South Africa, and India.

Woglum, in his interesting monograph on 'The Fumigation of Citrus Trees,' published in 1911, writes : ' The ease with which this gas may be generated as well as its destructive power, greater than that of any other known insecticide, leads the writer to believe that as soon as the various uses to which this gas may be put have been thoroughly investigated and placed on a stable basis the future development of hydrocyanic acid gas fumigation will be quite as important and extensive as has been its past development.'

The advantages to be derived from the use of hydrocyanic acid gas as a disinfectant for plague-infected houses and ships have been explained at some length in a paper by Liston, Stevenson and Taylor which was read at the All-India Sanitary Conference held in Lucknow and published on pages 162-175 of Vol. V of the proceedings of the conference which were issued as a supplement to the *Indian Journal of Medical Research*, 1914. Attention was drawn in this paper to the haphazard manner in which disinfection is sometimes practised. In many cases disinfection has become a ritual of whitewashing, spraying disinfectants on floors and walls, and applying deodorants to mask bad smells, methods which can have little effect on the germs it is desired to kill. The natural disinfecting processes, which arise from the competition between saprophytic and pathogenic bacteria, and from the devitalising effect of light and desiccation, and which are continuously and everywhere at work in nature, are often overlooked and neglected. These natural disinfecting processes bring about the rapid destruction of pathogenic bacteria like plague bacilli when they leave the body of a living animal. Disease-producing germs are killed by these means even within the body of an animal after death. Methods of disinfection, which do not take advantage of these natural disinfecting agencies and are not particularly directed against the disease-producing germs in the special situations where they are protected against these agencies, are wasteful and ineffectual.

Bubonic plague, for example, is maintained by the transfer of plague bacilli from rats to fleas and from fleas to rats. The bacilli are protected from the destructive effects of desiccation and light, from competition with saprophytic bacteria and even from the action of so powerful a germicide as perchloride of mercury, in the bodies of these living animals and insects. The essential point therefore to be aimed at in plague disinfection is the destruction of rats and rat fleas. If these are killed the plague bacilli will be destroyed

rapidly and effectively by the natural disinfecting agencies referred to above.

While these remarks refer to plague in particular, they can be applied in a modified form to a number of diseases which, like plague, are conveyed to man by 'porters' or 'intermediary hosts.' In this group of diseases, in addition to Bubonic Plague, may be placed Typhus Fever, Relapsing Fever, Ictero-hæmorrhagic Jaundice, Rat-bite Disease, Yellow Fever, Malaria, Dengue, Sand-fly Fever and certain other diseases in which the agency of a porter or intermediary host has been less definitely established.

The methods adopted for combating the plague in India are based on these principles. Chemical disinfection is now rarely practised. Apart from indirect methods of fighting the disease, as for example by decreasing the number of rats living in any place, by reducing the amount of food and shelter available for these animals, or by increasing men's resistance to the disease by inoculating them with anti-plague vaccine, the spread of plague is combated by catching rats in traps or by killing them with poison. Rat fleas are destroyed by using oily contact insecticides such as kerosene oil emulsion or hydrocarbon emulsion, or Cunningham's method may be employed of laying articles suspected of harbouring these insects on a layer of sand exposed to the heat of the sun. These methods nevertheless are imperfect in so much as they do not reach and kill, at one and at the same time, the rats and their fleas in the inaccessible places where they live. A poison which will do this satisfactorily must be of a gaseous nature and, for this reason, for some years, the present writer has been engaged, in co-operation with his colleagues at the Bombay Bacteriological Laboratory, in the study of such gaseous, vermin-destroying agents as sulphur dioxide, carbon monoxide, carbon disulphide, petrol vapour, formaldehyde gas and latterly hydrocyanic acid gas. Papers by Gloster, Stevenson, Taylor, Gore and Liston, dealing with these subjects, have appeared in the Scientific Memoirs of Officers of the Indian Services and have been read at a number of Sanitary and Scientific Congresses held in India since 1910. These studies have shown that hydrocyanic acid gas possesses many advantages over the other gases experimented with. Some of the numerous experiments on which this opinion is based are given in detail in the paper read at the Lucknow Conference. The following conclusions have been drawn from these and other experiments:

(1) Hydrocyanic acid gas is an effective disinfectant for Bubonic Plague and certain other diseases transmitted by porters or intermediary

hosts. The disinfecting action of this gas is not due to the direct action of the gas on the germs which cause these diseases but to the fact that it kills the animals or insects which harbour these germs and protects them from the natural agencies which bring about their destruction.

(2) Owing to its characteristic odour, and to the existence of simple and delicate chemical tests for its detection, this very poisonous gas can be used safely by persons who exercise a moderate amount of caution.

(3) In addition to these important advantages the gas does not injure the most delicate fabrics or metals; it does not render food unfit for consumption; grain will germinate after exposure to the gas.

(4) Heat is not required for generating the gas so that the danger of fire is avoided and the gas is lethal for vermin in so low a concentration when mixed with air that it is not then explosive.

(5) The chemicals for generating the gas are easily and cheaply obtained.

(6) The boiling point of hydrocyanic acid is  $26.5^{\circ}\text{C}$ . at 760 mm. The gas is rather lighter than air, having a vapour density of 0.959 at  $31^{\circ}\text{C}$ . The vapour pressure of hydrocyanic acid at  $13.25^{\circ}\text{C}$ . is 472 mm. The gas is therefore very diffusible, it can rapidly penetrate between grain bags and other cargo where rats find shelter. It even penetrates for some inches into grain stored in bags or spread upon a floor; it is easily and rapidly removed from rooms or holds by free ventilation.

(7) The quantity of gas required for effective disinfection depends on the air tightness of the space to be treated; the time the gas is allowed to act; the thoroughness of the distribution or convection of the gas; the cubic capacity of the space to be treated; whether the space is full or empty and the nature of its contents. Rats are killed in half an hour when exposed to a concentration of 20 parts of hydrocyanic acid gas in 100,000 parts of air, but some insects such as lice are killed only after a longer exposure, six hours, at a higher concentration (80 to 100 parts of HCN per 100,000 parts of air). The length of exposure and the concentration of the poison required will depend on the object to be attained. In general, given a compartment which can be made reasonably air tight, one half to three quarters of an ounce of potassium or sodium cyanide will suffice for one hundred cubic feet of space and the gas should be allowed to act for from four to six hours.

The experiments detailed in the paper referred to above were made with a machine which is illustrated in the paper and the method of using the machine was demonstrated at the Conference. The machine was

constructed of such materials as were procurable in India and it was only intended to be used for experimental purposes. Since that machine was made a number of other machines have been constructed on the same principles, *viz.*, the generation of the gas by the mixing of a solution of sulphuric acid with a solution of potassium cyanide in a closed chamber placed in the open air. The gas is propelled into and drawn out from the space under treatment by means of a fan and pipes. Modifications of a minor kind have been made in the new machines according to the nature of the work required of them. With these machines plague-infected houses have been fumigated in Bombay and Poona cities.



Type of machine used in Bombay City.

Railway carriages infested with bugs have been cleared of these pest for the Great Indian Peninsula Railway. Portions of a plague-infected hospital ship, which could not be treated with Clayton gas, because of the danger of fire, were fumigated with success. Hospitals in Bombay which were infested with bugs, were treated with the gas. A plant was erected and used at the Alexandra Docks, Bombay, for the treatment of the infested clothing and kit of soldiers and followers during the war.\* The experience gained in this way, although unattended by any kind of

\* Description and plans of this plant are reproduced at the end of this paper.

accident, naturally revealed defects which had to be overcome before fumigation with hydrocyanic acid gas could be recommended for general use. These defects, so far as they are known, have been eliminated in the latest type of machine which I have had constructed during the period I have been placed on deputation in England. Before describing this new machine I propose to review the experience of some other workers in this field of research.

The very important monograph on fumigation with hydrocyanic acid prepared by the United States Agricultural Department Bureau of Entomology, Bulletin No. 90, published in three parts in 1911 and written by R. S. Woglum and C. C. McDonnell, has been referred to. Woglum, in part 1, gives elaborate details of the methods adopted for fumigating Citrus trees in California. The usual method of generating the gas consists in adding solid lumps of cyanide to a 1 to 2 or a 1 to 3 solution of sulphuric acid in water. The quantity of sulphuric acid and cyanide used varies with the size of the tree and the character of the insect pest to be destroyed and is calculated by means of certain tables given in the text. The tree is first covered with a specially prepared tent, the water and acid are filled into an earthenware pot of about two gallons capacity and capable of holding twenty ounces of cyanide. This jar, charged with acid, is placed within the tent, then the cyanide is added, and the tent is immediately closed. Very rapid and considerable loss of the gas results if the tent is not made of suitable material. Woglum writes (page 11, *ibid.*): 'The results depend directly on the tightness of the cloth, in fact this consideration of tightness of tenting is one of the most important factors in the entire fumigating procedure. On it depends not only the efficacy of the treatment but also to some extent the cost of the operation. A dosage recommended as securing certain results with tents of a given degree of tightness will not produce the same results with tents of less closely woven material. Even although the initial cost is greater, tightly woven material is the most economical in the long run.'

Some idea of the extent to which fumigation with hydrocyanic acid is practised in California can be gathered from the fact that so great is the demand for fumigation tents that several concerns make a special business of meeting it.

In discussing the dangers associated with the use of this gas Woglum writes (page 80, *ibid.*): 'Hydrocyanic acid gas is one of the most deadly gases so that considerable care is necessary in its use. Such exaggerated



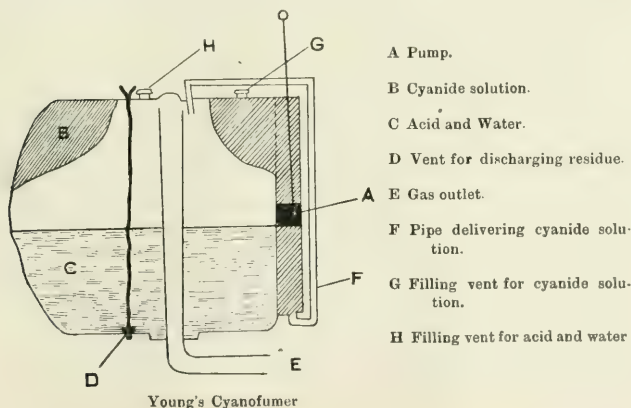
cautions have been written in view of its poisonous properties that the public at large have come to believe that a single whiff of the gas will produce the immediate death of an individual. This erroneous idea should be corrected. A whiff of the gas will not cause immediate death, neither will two or three whiffs. If subjected to a strong gas for a minute or two, undoubtedly a person would be overcome. The writer has never yet had a record of a person killed by hydrocyanic acid gas while fumigating. In California, men work around tents every night for weeks at a time without any ill effects. During these operations they are breathing the gas in a dilute form much of the time. These experiences are mentioned not with the idea of tempting people to be careless in the use of this gas, but merely to correct the erroneous conception that a whiff of the gas will cause instant death. This gas is most dangerous and the writer has seen men who were subjected to a great strength of it for several minutes at a time overcome by its effects, although they revived later. If the proper precautions are taken the careful operator will run no risk whatever.'

'McDonnell's portion of the bulletin deals with the chemistry of fumigation. He concludes from the experiments he records, that the presence of chlorid or nitrates in cyanides which liberate hydrochloric and nitric acid respectively, together with hydrocyanic acid, on treatment with sulphuric acid cause very marked decomposition of the hydrocyanic acid. The effect produced by hydrochloric acid is much more marked than that produced by nitric acid. In one case over 92 per cent of the hydrocyanic acid was decomposed and only a little over 7 per cent evolved. This is a larger amount of sodium chloride than would ever be found in a commercial sample, but it shows the important bearing this impurity has upon the results. Practically all commercial potassium and sodium cyanides contain sodium chloride in greater or less amount. Potassium cyanide is frequently sold as "98-99 per cent pure" which in reality is a mixture of potassium cyanide, sodium cyanide, and sodium chloride and on analysis may show even 100 per cent expressed as potassium cyanide yet there may be several per cent of sodium chloride present. For fumigation work an analysis of a cyanide is of little value unless the chlorine content is also determined.'

It will be convenient here to refer to a paper published some years later by H. D. Young, as it deals, like those mentioned above, with the use of hydrocyanic acid gas in agriculture (Circular No. 139, University of



California, College of Agriculture). This paper contains a description of a portable machine for generating hydrocyanic acid for the fumigation of fruit trees. Young criticises the pot method of generating hydrocyanic acid used by Woglum because he says 'there is greater or less amount of injury done to tents by spilling of sulphuric acid or dragging tents through acid residue, this not only greatly increases the expenses of fumigation but it impairs the efficiency of fumigation because of the greater tent leakage caused by small holes which are overlooked.' 'Machines,' he argues, 'should almost entirely eliminate tent burning since acid is not handled for each tree and since the residue can be disposed of in a safe place.' The poisonous and corrosive nature of this residue must not be forgotten; it consists of potassium and sodium sulphate, sulphuric acid, and some hydrocyanic acid. The machine which Young recommends is known as the 'Cyanofumer.' It consists of an iron reservoir in the bottom of which is placed a strong solution of sulphuric acid. Around the upper part of the reservoir a circular chamber contains a solution of potassium or sodium cyanide. Connected with this upper chamber is a pump by means of which successive measured quantities of cyanide solution can be pumped through a lateral pipe opening into the top of the reservoir above the acid solution. When the cyanide solution falls into the acid solution hydrocyanic acid gas is generated. This gas escapes by a pipe which opens above the acid solution and passes through the bottom of the reservoir. The accompanying diagram explains the mechanism.

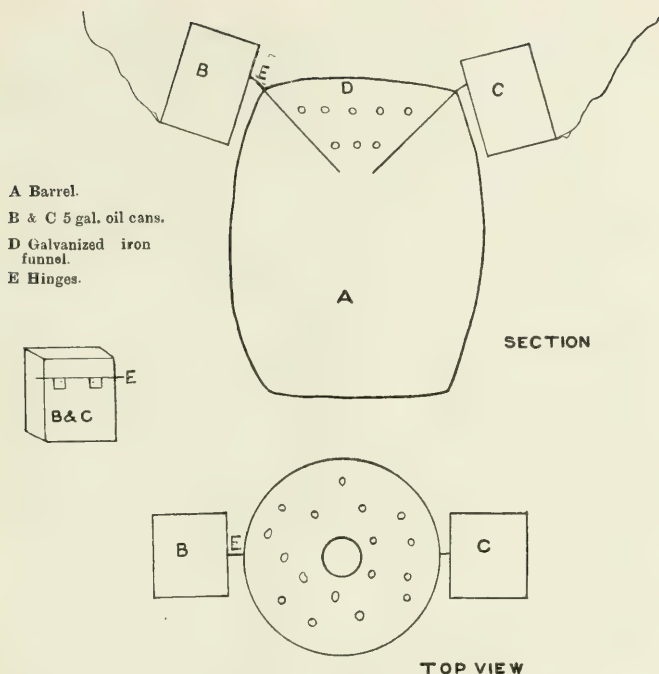


Young concludes his paper with the following remarks: 'The principle advantages of machine fumigation in general are greater accuracy of dosage, cleanliness, rapid generation, so that greater concentration of gas under the tent is obtained and a lessening of tent burning. This probably constitutes the greatest saving of the new methods. The Cyanofumer introduces an entirely different ratio in the dosage schedule since successive quantities of sodium cyanide are added to a large amount of sulphuric acid. The amount of sulphuric acid, water and cyanide recommended for use give a higher and more uniform production of gas under the proper conditions. The best production of gas is produced with a high temperature.'

N. Roberts seems to have been the first to use hydrocyanic acid for the fumigation of ships. He described the method used in New Orleans in the Public Health Reports, Washington, No. 50, dated 11th December, 1914, pages 3321-3725. The apparatus used seems to have been a modification of the 'pot' method of Woglum known as 'the dumping fixture' presently to be described. Apparently Roberts found some difficulty in dealing with the poisonous residue after the hydrocyanic acid gas had been evolved for he added to the container a strong solution of sodium carbonate with the object of 'rendering the waste less poisonous, corrosive and troublesome.'

! But the use of this gas for the fumigation of ships in America has been more fully reported by R. H. Creel in two of the United States Public Health Reports, *viz.*, Reprint No. 313, dated 3rd December, 1915, 'Hydrocyanic Acid Gas, its Practical Use as a Routine Fumigant' and Reprint No. 423, dated 7th September, 1917, 'Rodent Destruction on Ships.'

The 'dumping fixture' described and illustrated in Creel's first paper consists of a barrel which is used to contain a solution of sulphuric acid. Boxes are hinged to the upper and outer margin of the barrel and in them is placed the cyanide. A rope is attached to the bottom of each box in such a manner that when it is pulled upon from above the box is tilted over the mouth of the barrel and the cyanide is deposited on a sloping and perforated tray which allows it to fall into the acid solution. The accompanying diagram explains the mechanism.



The dumping fixture.

Creel's first paper also contains a copy of the instructions to officers of the Public Health Service, U.S.A., which are to be followed in the fumigation of vessels with hydrocyanic acid gas and are reproduced here.

'When performing the cyanide fumigation of compartments you will be guided by the following minimum requirements:—

1. On account of great danger to human life from hydrocyanic acid gas, specific arrangements should be made for the disposition of the crew during the fumigation process, especially if one or two compartments of a vessel are to be treated. A written statement must be obtained from the captain or first officer of the vessel that the latter is ready for fumigation, and that every member of the crew has been

accounted for, as not being in the vessel or else not exposed to the fumes of the gas. Persons in one compartment have been killed by fumes escaping from another compartment undergoing fumigation.

2. Not less than 5 ounces of potassium cyanide or  $3\frac{3}{4}$  ounces of sodium cyanide shall be used to each 1,000 cubic feet of space, inclusive of that occupied by cargo.

3. To each ounce of potassium cyanide 1 fluid ounce of commercial sulphuric acid 66 B. and  $2\frac{1}{2}$  fluid ounces of water shall be used.

4. To each ounce of sodium cyanide  $1\frac{1}{2}$  fluid ounces of commercial sulphuric acid 66 B. and 2 fluid ounces of water shall be used.

5. All ingredients shall be weighed and mixed immediately prior to each fumigation.

6. All parts of the vessel shall be placed under fumigation simultaneously except such compartments as may not require fumigation in the opinion of a representative of the United States Public Health Service.

7. All compartments placed under fumigation shall be kept closed for not less than one hour.

8. The hatches of 'tween decks and the doors of sub-compartments are to be opened prior to fumigation and the barrel or other generator is to be placed so as to secure the most rapid and efficient diffusion of the gas.

9. All work is to be done under the supervision and to the satisfaction of the medical officer in charge, United States Public Health Service, or his representative.

Tables given in Creel's second paper, dated 7th September, 1917, show that, at that time, as many as 182 vessels had been treated with hydrocyanic acid gas. The result of the fumigation of these vessels with this gas is compared with the result of the fumigation of 62 vessels treated with sulphur dioxide in the following table I.

TABLE I.

Gas used for fumigation.	Number of vessels treated.	Number of rats killed.	Number of rats trapped.	Percentage Efficiency.
Sulphur dioxide . . .	62	747	223	77
Hydrocyanic acid . . .	182	2,811	121	95

In explanation of this table it should be noted that the period of exposure to sulphur dioxide gas was six hours, but to hydrocyanic acid gas only  $1\frac{1}{4}$  hours. After the ship had been fumigated rat traps were set in the ship. It was found that fumigation, as carried out, never killed all the rats on the ships for various reasons but chiefly because the whole ship was never treated at one time. In order to estimate the rat infection of the ship and the efficiency of fumigation the number of rats found dead as the result of fumigation were added to the number of rats caught in traps after fumigation: the percentage efficiency being expressed as a proportion between the rats killed by fumigation and the total rats killed and caught added together.

In another table Creel compares the result of fumigation with the two gases in different parts of the ships. He endeavours to measure, for example, the efficiency of fumigation with the two gases when only the superstructure of the ship is considered. The superstructure of the ship includes store-rooms, crews' quarters, cabins and poop deck. He also considers the result of fumigation on empty and laden holds.

TABLE II.

Gas used for fumigation.	Number of vessels.	Compartment of vessel considered.	Number of rats killed.	Number of rats trapped.	Per cent Efficiency.
Sulphur dioxide .	32	Superstructure.	132	107	55
Hydrocyanic acid .	31	Superstructure.	729	45	94
Sulphur dioxide .	28	Hold empty	702	28	96
Hydrocyanic acid .	34	Hold empty	854	9	99
Sulphur dioxide .	10	Hold loaded	104	59	64
Hydrocyanic acid .	10	Hold loaded	80	20	80

This table shows that in all cases hydrocyanic acid gas is more efficient than sulphur dioxide; the relative efficiency of hydrocyanic acid is most marked when the superstructure is considered. In this connection it is important to note that this is just the part of a ship which

is most often infected with plague and the part which cannot be treated with sulphur dioxide without causing much damage both to the food and fittings. For example, Dr. Willoughby, the Medical Officer of Health Port of London, in a paper on 'The Course of Plague on Ships' read at the 30th Annual Congress of the Royal Sanitary Institute and published in the *Medical Officer* of the 18th October, 1919, in describing his experience of plague on board five ships infected in Bombay and which came to London during the years 1917, 1918 and 1919, shows how infection was associated with those parts of the ship classed by Creel as 'superstructure,' viz., the food store-rooms and crews' quarters. He expresses the opinion 'that fumigation must not omit the inhabited parts of the ship and should include the food store-room which is usually close at hand. These places may, with advantage, be fumigated without the holds, but never the holds without the living quarters.'

In recording his experience of the use of hydrocyanic acid gas for the fumigation of ships Creel makes no mention of any accidents attending these operations. There can be no doubt however that the method of generating this gas by means of 'the dumping fixture' is associated with danger, first because the sudden evolution of large volumes of gas give rise to high concentrations of it in the immediate neighbourhood of the generator, and second because no special measures to distribute the gas or remove it from the ship, other than those mentioned in paragraph 8 of the United States Public Health Department's instructions to officers, are taken. Pockets of gas are liable to form and remain in the ship when fumigation is supposed to be completed. Accidents due to these defects have occurred as the following narrative shows: 'The S.S. *Devonian* arrived in quarantine at Boston from Liverpool on the 31st October, 1916, and, on account of the reported outbreak of plague at Liverpool, the process of fumigating the vessel, apparently with the object of destroying rats, was carried out by the United States Public Health Department. The agent employed was hydrocyanic acid gas which was turned into all the holds of the ship. On the following morning the fumigation was completed and permission was given for opening the holds and discharging the cargo. On the morning of November 2nd, six carpenters went down into the lower hold, which was empty, to adjust the shifting boards for a grain cargo, and soon after an alarm was raised that some of them had been overcome by gas. The chief officer was called, and, accompanied by a lamp trimmer, went to their

rescue with cloths covering their heads. They found the carpenters at the foot of the ladder in a state of prostration and proceeded to tie them to ropes in order to have them hauled up. One of the men was hauled up in this way when the chief officer and the lamp trimmer were themselves overcome by the fumes and became unconscious. Another of the crew now went down, with a gas helmet on, and had all the men hauled up on deck. Three of the carpenters subsequently succumbed to the fumes and the other three, as well as the chief officer, remained for several weeks in serious danger of their lives but eventually recovered.'

On another occasion 'the S.S. *Galileo* arrived from Hull in quarantine in New York on the 24th October, 1916, and was fumigated under the supervision of the Health authorities, the agent employed being hydrocyanic acid gas. All hatches were removed for ventilation at 10-30 A.M., and two hours later, the Master and several officers went into the various spaces to see if the fumes had cleared away. At 2-10 P.M., as the vessel was entering the dock, a fireman was found unconscious in the starboard bunker pocket. He was taken on deck and efforts made to restore him but the doctor, who arrived in a short time, pronounced the man dead.'

These and perhaps similar accidents have led the United States Public Health authorities to devise special means for getting rid of the poison gas after fumigation, for, in Reprint No. 428, dated October 1917, Grubbs writes on 'Ventilation after Fumigation.' In this paper he refers to a personal communication from Dr. Heiser who had seen a machine we used in Bombay when on a visit to our Laboratory. It appears from the text of Grubbs' paper that he did not grasp the details of the procedure adopted in our machine. He proceeded to devise a method of getting rid of the gas by means of a machine which is quite independent of the generator and is used after fumigation has been completed. His machine consists of a large propeller, 32 inches in diameter, driven by a two-cylinder, two-cycle, air cooled, three horse power petrol engine at 1,600 revolutions per minute and capable of discharging air at the end of a muslin chute, 28 inches in diameter and 20 feet in length, at the rate of 8,340 cubic feet per minute. The muslin chute is lowered into the hold while the fan is fixed at the deck level, when the motor is started air is driven down the chute and displaces the poison gas. The chute is only suitable for use in empty holds. The employment of this machine in such cases effects a decided improvement on the previous



methods of fumigation and materially lessens the chance of accidents, but the machine has a limited application only.

In a paper by C. E. Corlette on 'Insecticidal Fumigation in Ships with special reference to the use of Hydrocyanic Acid and the Prevention of Ship-borne Yellow Fever,' published in the *Medical Journal of Australia* of November 4th, 1916, reference is made to the danger associated with the use of hydrocyanic acid gas in the following terms: 'It certainly is a very remarkable fact that, despite the poisonous character of the fumes, and the varying intelligence and carefulness of users, no deaths seem ever to have occurred in New South Wales from this cause. According to R. S. Woglum, California has been equally fortunate. I have heard that, in South Africa, at least in earlier days, there were some fatal accidents among Kaffirs, and I have heard of one or two narrow escapes on board ship at Sydney. The drunken sailor or fireman is the usual cause of worry. Unless every place is locked up and careful inspection carried out beforehand, a drunken man may get in and be killed. Although it appears that no people have been killed in New South Wales, I have heard of two accidents with fatal results in other Australian States. In one, after an expert has refused to do a ship under certain circumstances which he did not consider safe enough, a quite unqualified and inexperienced experimenter took it upon himself to carry it out. After it was done, and the space opened up, the dead body of a man was found within. This was in West Australia. The other accident occurred in Queensland. Here some ignorant and foolhardy person essayed to rid the lower rooms of a house of bugs by fumigating with hydrocyanic acid. Two men were left sleeping in a room upstairs, and were afterwards found dead, a result which might have been expected. No potent fumigant can possibly be made entirely foolproof. It should not be forgotten that, in spite of all precautions, a large number of men are killed every year by accidents which occur in connection with the working of cargo. This toll of life is taken in the production of wealth. If that be justifiable, it is also worth while incurring a little risk, really a much smaller risk in its totality of effect, if it will insure a country against the serious loss of wealth, to say nothing of life and health, which would take place with the introduction of an epidemic.'

A careful perusal of the available literature on the subject shows that very few, if any, accidents have occurred when the gas is generated in the open air, for example when the gas is used in connection with the extensive practice of fumigating Citrus trees in California or oranges in Australia

and that the majority of accidents, which undoubtedly have occurred, have been associated with ships, houses, or closed spaces. The explanation of this important and strikingly different experience in the use of the gas in the two cases can be attributed to the fact that, in the open air, it is difficult to obtain a sufficient concentration of the gas to poison men. This is in accord with the experience gained during the war, for, when poisonous gases were first used, attention was soon directed to hydrocyanic acid gas. The French Government, in particular, carried out many experiments with this gas and found that it would not kill men in the open or in trenches. Man, as compared with other animals, is relatively less susceptible to the poison. Birds, especially hens and sparrows, are the most susceptible of all animals to the action of hydrocyanic acid gas. Of mammals, a dog is killed when exposed for half an hour to eight parts of the gas in 100,000 parts of air, cats require twelve parts, rabbits fifteen parts, rats twenty parts, goats and monkeys twenty-five parts per 100,000 parts of air for half an hour to kill them. Man probably requires as much as a monkey or goat to kill him. In low concentration the gas causes a very disagreeable sensation in the throat and eyes, and, in these small and harmless concentrations, would be avoided by persons brought in contact with it. A man does not become unconscious till higher concentrations are attained and, if the concentration of the gas is not increased, there is, in these circumstances, a comparatively long latent period before death supervenes. A person who is thus rendered unconscious rapidly recovers when placed in the open air; the gas, in fact, acts as an anæsthetic. In still higher concentrations, however, the gas causes almost instant death, probably through its action on the heart. The whole effect of hydrocyanic acid gas on men may be appropriately likened to the effect of chloroform on them.

The most important deduction to be drawn from the experiences of those who have used this gas is that it can be used safely if generated in a machine placed in the open air, and, it follows, that the 'pot method' and 'dumping fixtures' should be avoided, especially when houses or ships have to be fumigated. Apart from the objection, that the pots or dumping fixtures must be placed within the space to be treated, these methods are objectionable because the gas is evolved with almost explosive rapidity, high, and therefore dangerous, concentrations of the gas are developed; the generation of the gas is in fact completely out of control as can be gathered, when it has been stated by a writer familiar with these methods, 'that ordinary speed in departing from the

room will likewise safeguard the operator in fumigations on land where the cyanide is dropped in by hand.' A third objection to the pot and dumping fixture method is the necessity of handling the corrosive and poisonous waste in a closed space ; in the open air the waste can be disposed of safely. The generation of the gas by mixing two solutions of cyanide and acid, as in our machine, or as used in Young's Cyanofumer, has the great advantages, not only that the gas can be generated in the open air, but the quantity and the rate at which the gas is evolved is completely under control, for the generation of the gas can be stopped or started at will and the waste can be disposed of without danger.

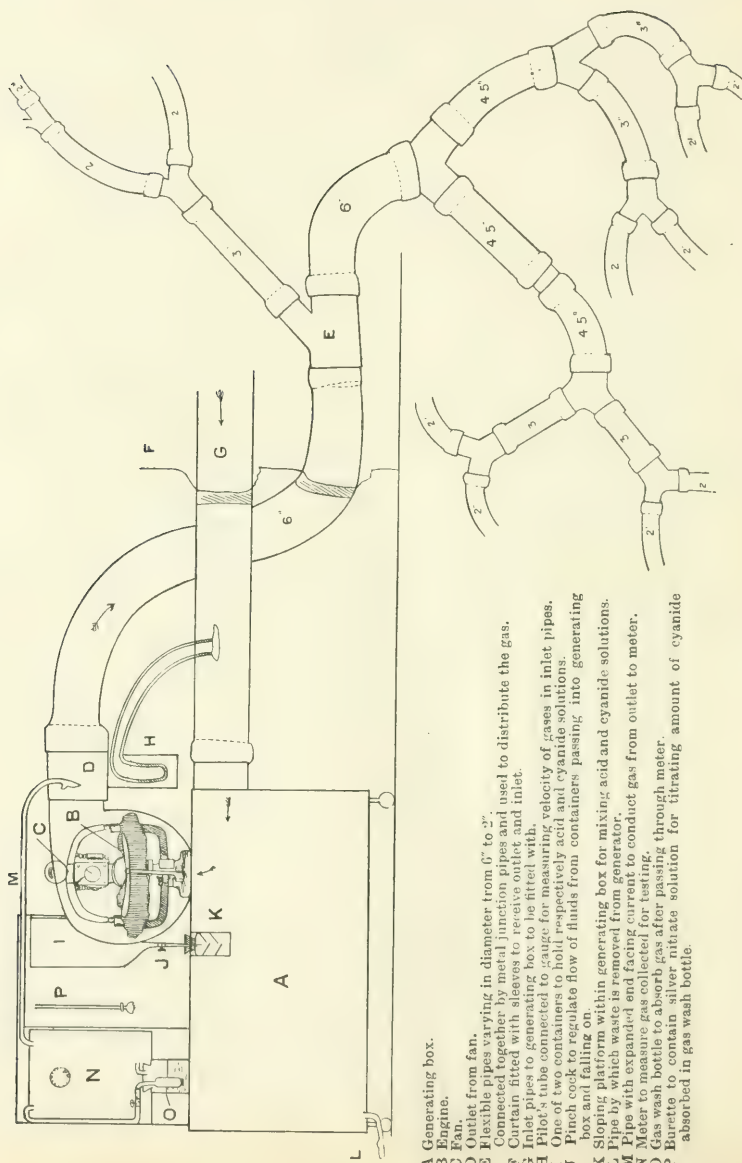
If a generator is used, which is placed in the open air, then arrangements must be made to convey the gas into the compartment or compartments which have to be treated. In Young's Cyanofumer a pipe carries the gas into the tent used to cover the tree ; the pressure of the gas as it is evolved is relied on to carry it into the tent. In our machine, and in any machine which must discharge gas into large spaces such as exist on ships or in houses, pipes of considerable length are necessary, and, in these circumstances, a fan is required to propel the gas along the pipes. There are other advantages to be obtained in using a fan for this purpose. Arrangements can be made to circulate the gas in the compartment under treatment so that the gas is better distributed. This is an important consideration where rapid and effective treatment is required. Moreover, by means of a suitable fan, it is possible to rapidly dilute the poison gas with air so that very high and dangerous concentrations of it are not developed even in the generator. An arrangement of this kind very greatly decreases the chance of accidents. By means of a fan, too, the poison gas can be easily displaced by fresh air when the period of exposure to the gas has been deemed to be sufficient. But perhaps the most important feature of our machine is the arrangement by which the approximate concentration of the poison gas contained within the compartment can be tested at any time. This conduces greatly to efficiency, economy, and safety for the following reasons. The volume of hydrocyanic acid gas evolved from a sample of cyanide depends, as has been shown by MacDonnell, on its composition ; also on the temperature of the acid mixture ; moreover, the concentration in a compartment varies, not only with the volume of gas delivered into it, but also on the amount which escapes from it or is absorbed by the contents of the compartment. When so many factors, not easily controlled, may cause

variations in the concentration of the gas in the compartment, it is obvious that any means of estimating the concentration of the gas in the compartment at any time will conduce to efficiency.

Alternatively, in order to allow for possible loss of gas, a much larger quantity is generated than is actually required, so that, if the concentration of the gas can be estimated during fumigation, economy will be effected. Safety will also be ensured for high concentrations will be avoided.

The general arrangements and the improvements effected in our cyanide fumigator are shown in the accompanying diagram where (A) is the generating box. (B) is a petrol motor used to drive a special fan (C). This fan is capable of delivering 1,200 cubic feet of air per minute along pipes at a pressure of six inches on a water gauge when the motor develops 3,600 revolutions per minute. A light armoured flexible hose pipe, six inches in diameter and six feet six inches long, is attached to the outlet from the fan at (D). Sections of collapsible pipe, made of a special light gas proof fabric, cut in sections nine feet in length, can be joined to the flexible hose at (E). Other sections of this collapsible pipe may be joined to this section and to one another by metal junction pieces. These junction pieces are so shaped as to connect either two pieces of the same diameter of tubing together or one piece of one diameter to two pieces each of a smaller diameter. In this way the pipe leading from the fan divides and subdivides. The gas is ultimately discharged into the compartment under treatment through eight or ten pipes, two inches in diameter, at points as far distant as possible from the place where the inlet and outlet pipes enter the compartment.

The compartment under treatment has of course been made as air tight as possible by covering all openings with paper or fabric. The outlet tube from the fan and the inlet tube to the generating box pass into the compartment through a fabric curtain (F), which is fitted with special sleeves to receive these tubes. This curtain can be fixed over an open door or window or other suitable opening. The inlet tube (G) conducts the air from the compartment to the generating box. Hydrocyanic acid gas is added to the air in the generating box and the mixed gases are then delivered through the fan and pipes back into the compartment again. The velocity of the mixed gases passing through the inlet tube can be measured by using a Pitot's tube and U-shaped water gauge. Knowing the diameter of the tube, the volume of



A Generating box.

B Fan.

C Engine.

D Outlet from fan.

E Flexible pipes varying in diameter from 6" to 3".

F Connected together by metal junction pipes and used to distribute the gas.

G Inlet pipes fitted with sleeves to receive outlet and inlet.

H Pilot's tube connected to gauge for measuring velocity of gases in inlet pipes.

I One of two containers to hold respectively acid and cyanide solutions.

J Pinch cock to regulate flow of fluids from containers passing into generating box and falling on.

K Sloping platform within generating box for mixing acid and cyanide solutions.

L Pipe by which waste is removed from generator.

M Pipe with expanded end facing current to conduct gas from outlet to meter.

N Meter to measure gas collected for testing.

O Gas wash bottle to absorb gas after passing through meter.

P Burette to contain silver nitrate solution for titrating amount of cyanide absorbed in gas wash bottle.

mixed gases delivered from the generating box into the compartment per minute can be calculated.

The hydrocyanic acid gas is generated by placing solutions of strong sulphuric acid and potassium cyanide each separately in one of two glass containers, one of which is shown at (I). The fluids in these containers are delivered into the generating box through rubber pipes connected to each container and to two glass tubes which pass through a rubber cork fixed in the lid of the generating box. On each rubber tube a pinch cock (J) is fixed. These pinch cocks, when properly adjusted, allow the fluid in each container to flow at an equal rate upon the surface of a sloping platform (K) placed beneath the rubber cork referred to above. The fluids are thoroughly mixed on this platform as they flow towards the side of the box, by the action of small baffle plates fixed to the surface of the platform. The gas is generated as the fluids mix and the spent fluid or waste collects in the bottom of the generating box. The waste can be removed from the box when desired through the pipe (L). This pipe is closed by a screw pinch cock applied to the piece of rubber tubing fixed to the end of the pipe.

The solutions of acid and cyanide are readily made in graduated bottles, using measured quantities of cyanide and sulphuric acid. The cyanide and acid are stored in the form of charges. Each charge of acid and cyanide weighs  $\frac{1}{2}$  a kilogramme and is made up with water to a volume of two litres. A double charge, *i.e.*, one of cyanide and one of sulphuric acid is sufficient under ordinary circumstances to fumigate three thousand cubic feet and should give, in a fairly air tight empty compartment, when a good sample of cyanide is used, one hundred parts of hydrocyanic acid gas in one hundred thousand parts of air. A sufficient number of charges are carried with each machine to deal with thirty thousand cubic feet. The machine may therefore be regarded as a unit capable of fumigating compartments measuring from thirty to forty thousand cubic feet. If larger spaces have to be treated two, three or more units will be required and can be used together. A Medical Officer in charge of a Port should be provided with at least ten units. The fumigation of a ship as a rule must be completed in as short a time as possible; the plan of using a number of comparatively small units greatly facilitates the work.

When the approximate concentration of the poison gas in a compartment is required to be known, either during fumigation or immediately before the compartment is entered, the generation of the poison gas is



suspended but the fan continues to circulate the air contained in the room through the generator. A sample of this circulating air can be drawn off through the pipe (M) from the outlet of the fan to the meter (N), when the tap on the pipe leading from the meter to the gas wash bottle (O) is opened. A solution of caustic soda, containing a little potassium iodide, is placed in the gas wash bottle.

One half a cubic foot of air from the room is passed through the wash bottle. In passing through the alkaline solution the acid is fixed while the air escapes. It is an easy matter then to estimate the amount of cyanide formed in this solution by Liebigs method. The standard solution of silver nitrate used is conveniently made up of such a strength (5.414 grammes of silver nitrate to the litre of water) that 1 c.c. of it is equivalent to 10 parts of hydrocyanic acid gas in 100,000 parts of air when half a cubic foot of mixed gas is tested. The solution is contained in the graduated burette (P) and is added to the soda solution with constant stirring till a permanent slightly yellow turbidity is obtained. The number of cubic centimetres of silver nitrate solution used to obtain this permanent turbidity is noted, and this number multiplied by ten gives the number of parts of hydrocyanic acid gas in 100,000 parts of the air in the compartment.

When the period of fumigation is completed pipe (G) is withdrawn from the room through the sleeve in the curtain (F) and is placed in the fresh air. The volume of fresh air now passing through the generator each minute is noted. The fan continues to revolve for so long as, and until, a volume of air has been passed into the compartment equivalent to the total capacity of the compartment. For example if it was found that the volume of air passing through the generator was 500 cubic feet a minute, and the total capacity of the compartment was 30,000 cubic feet, the fan would continue to work for one hour when 30,000 cubic feet of fresh air had been pumped into the compartment. Another test of the concentration of the gas in the compartment should then be made by replacing the inlet pipe (G) through the curtain (F) and the circulation of the air of the compartment through the generating box is resumed. Meanwhile half a cubic foot of this air is passed through the meter and gas wash bottle. The quantity of hydrocyanic acid absorbed is titrated as before with the silver nitrate solution.

The fan, engine, and chemical cabinet are firmly fixed to the lid of the generating box which is held in position by means of lugs and screws. An air tight joint is secured by a band of rubber which is fixed on the rim



of the box. When the screws are loosened the lid can be removed and all the parts of the machine fixed on the top of it are so arranged that, when inverted, they occupy the interior of the generating box. In this position the fan, engine, etc., are protected from damage when the machine is moved from one place to another.

A second, rather shallower box, but otherwise of exactly the same dimensions as the generating box, contains all the chemicals, fabric tubes, metal junction pieces, pails, paste, paper, etc., which are required in carrying out fumigation. This box is placed over the inverted lid of the generating box and is held in position there by the screws fixed to the sides of the generating box. The screws are now adjusted to the lugs which are fastened to the bottom of the equipment box.

The flexible armoured hose pipes, which consist of two pieces six inches in diameter, two pieces four and a half inches in diameter, two pieces three inches in diameter and two pieces two inches in diameter, all six feet six inches in length, form two bundles, when those of smaller diameter are fitted into those of larger diameter. The large diameter pieces serve as inlet and outlet pipes, while those of smaller diameter are used when sharp corners have to be turned in laying the tubing in the compartment under treatment. Each of the bundles is placed in a separate box which is affixed to the side of the generating box. These boxes are used as handles for lifting the whole machine as a stretcher is lifted by the poles on either side; they can be readily detached from the generating box when required. The whole machine can thus be lifted and transported by two men; it weighs between three and four hundred-weights.

#### A BRIEF DESCRIPTION OF THE PLANT ERECTED AT THE ALEXANDRA DOCKS, BOMBAY, FOR FUMIGATING KIT, ETC., WITH HYDROCYANIC ACID GAS.

A PORTION of one of the sheds for storing cargo at the Alexandra Docks, Bombay, was shut off by temporary brick walls and a roof so as to form a room 34 feet long, 16 feet wide and 10 feet high. Two of the walls of this fumigating room, marked A and B in the plans, were formed by a portion of the permanent stone walls of the shed. Four windows were fixed in these walls, as is shown in plan 3. Three of the windows opened into the room at the level of the roof of the room, while the fourth, the middle one in wall A, plan 3, opened at the level of the floor,

These windows could be opened or closed from the outside of the building.

Two of the walls of the room, C and D in the plans, were constructed of bricks. The room was entered by a door, situated at one end of wall D. The generating and testing apparatus was fixed to the middle of the wall. (*See plan No. 1.*)

The roof of the room, marked E in the plan, was only 10 feet above the floor level.

The room contained stands, these were fitted with pegs and provided with a perforated shelf. Clothes and blankets could be suspended on the pegs; while books, boots, brushes, etc., could be placed on the shelf. The stands were erected in three rows parallel to the length of the room. Plan 2 is a cross section through the room; while plan 3 shows a plan and elevation of the room and stands.

Below each stand square wooden pipes were fixed. These are shown in dotted outline in plan 3. They run along the whole length of each stand, and, at one end, enter the generating chamber, the other end was closed. Holes, placed 1 foot apart, were bored in the upper surface of the pipes.

The generating chamber is situated at one end of the room. It was fixed to the wall. A section through the chamber is shown in plan 4, and a view of the chamber with the front removed is shown in the upper drawing, plan 5. Situated behind the generating chamber, on the outside of the fumigating room, is an electric fan. This fan is enclosed in a wooden case. The case is fixed to the wall and is provided with two sets of doors and a transverse partition which can be raised or lowered by a cord passing through the top of the case. Two circular openings in the brick wall D lead from the wooden case into the fumigating room. The upper opening passes directly into the room above the generating chamber. The lower opening leads into the generating chamber thence, along the wooden pipes, into the room through the holes in the pipes. The electric fan is placed immediately on the outside of this lower opening.

When the room is used for fumigating, the windows and doors are closed. The transverse partition in the wooden case containing the fan is raised, the doors of this case are also closed. When the fan is set in motion, air is drawn by it from the room through the upper opening and is discharged into the generating chamber through the lower opening. From the generating chamber the air passes along the wooden pipes

escaping into the room through the holes in the pipes beneath the stands. The air, in this way, is circulated from the room, through the fan, back into the room, as is illustrated in the upper drawing of plan 4. Another small box is situated above the wooden case containing the fan. This box contains two glass vessels ('irrigators'), one is used for a solution of sulphuric acid and the other for a solution of potassium cyanide. These glass vessels are connected by rubber tubing with two glass tubes which pass through the roof of the wooden case containing the fan and through a small hole in the wall D into the upper part of the generating chamber within the fumigating room. (See plan 5.) The glass tubes open over a lead-lined channel which passes, in a zig-zag fashion, along the back wall of the generating chamber to an outlet pipe connected with a drain outside the room. The flow of the solutions contained in the irrigators is controlled by two pinch cocks applied to the rubber tubing connecting the irrigators with the glass tubing. The fluids flowing down the glass tubes mix on the lead-lined platform within the generating chamber. The thorough mixing of the two fluids is facilitated, as they flow to the bottom of the generating chamber, by small baffle plates fitted on the lead-lined channel. Hydrocyanic acid gas is generated within the chamber when the acid and cyanide solutions mix. If the fan is working and the plant is arranged as is shown in the upper drawing in plan 4, the hydrocyanic acid gas is mixed with air and circulated through the fumigating room.

The concentration of the hydrocyanic acid gas contained within the room can be ascertained at any time by means of the apparatus illustrated in the bottom drawing, plan 5.

A measured volume ( $\frac{1}{2}$  a cubic foot) of the mixed gases in the room can be drawn by the aspirator Q, along the glass tube O, through the wash bottle P. A solution of caustic soda or potash is contained in the gas wash bottle, this solution absorbs any hydrocyanic acid gas in the mixed gases as they bubble through the bottle. The quantity of cyanide in the alkaline solution in the gas wash bottle can be determined by means of burette R, containing a standard solution of silver nitrate. The whole equipment for testing is contained in the cupboard marked 3 in plan 1.

When the period of exposure to the gas has been completed, the windows of the room are opened, the transverse partition in the fan case is lowered, and the doors at the back of the fan are opened. The fan, still continuing to work, now draws fresh air through the door, drives the

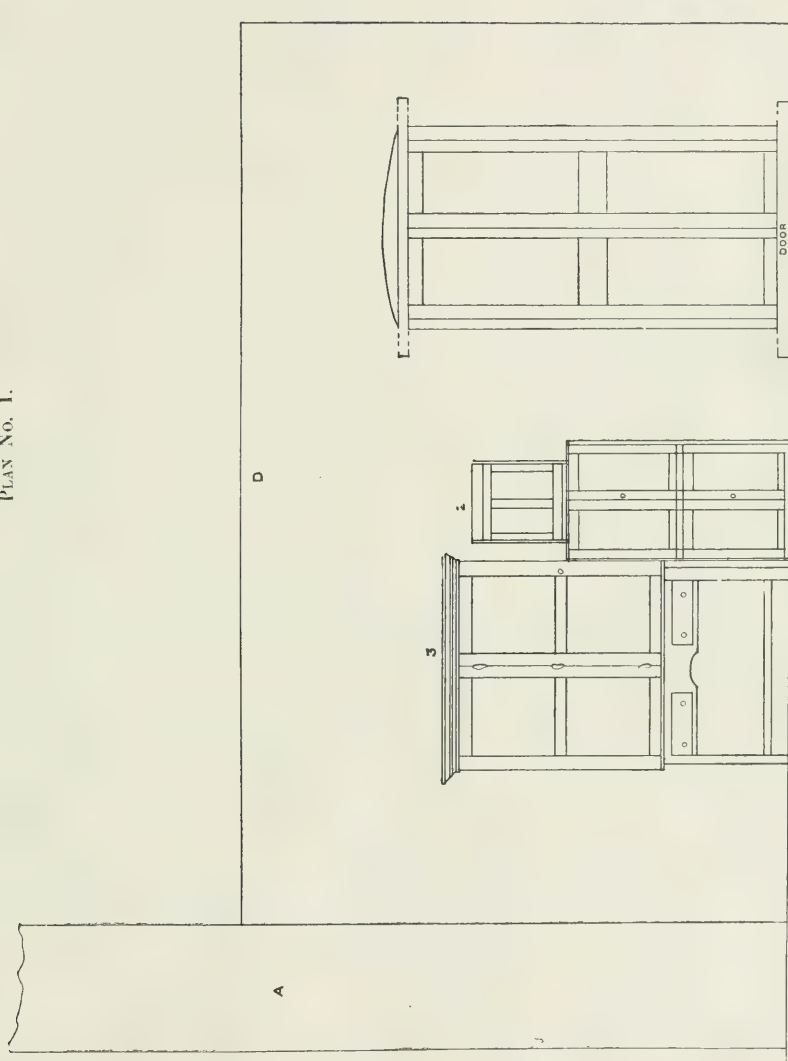
fresh air into the generating box along the pipes through the holes into the room and then out of the windows as shown in the lower drawing, plan 4. The room is in this way rapidly cleared of the poisonous gas and can be entered in a few minutes.

The room was originally constructed to accommodate the kit of about 180 men, each man being provided with 1 foot of space on the stands. Subsequent experience showed that the space provided was not sufficient for the kit of each man and the number of kits treated at one time was, therefore, reduced to 72, so that each man had  $2\frac{1}{2}$  feet of space on the stands.

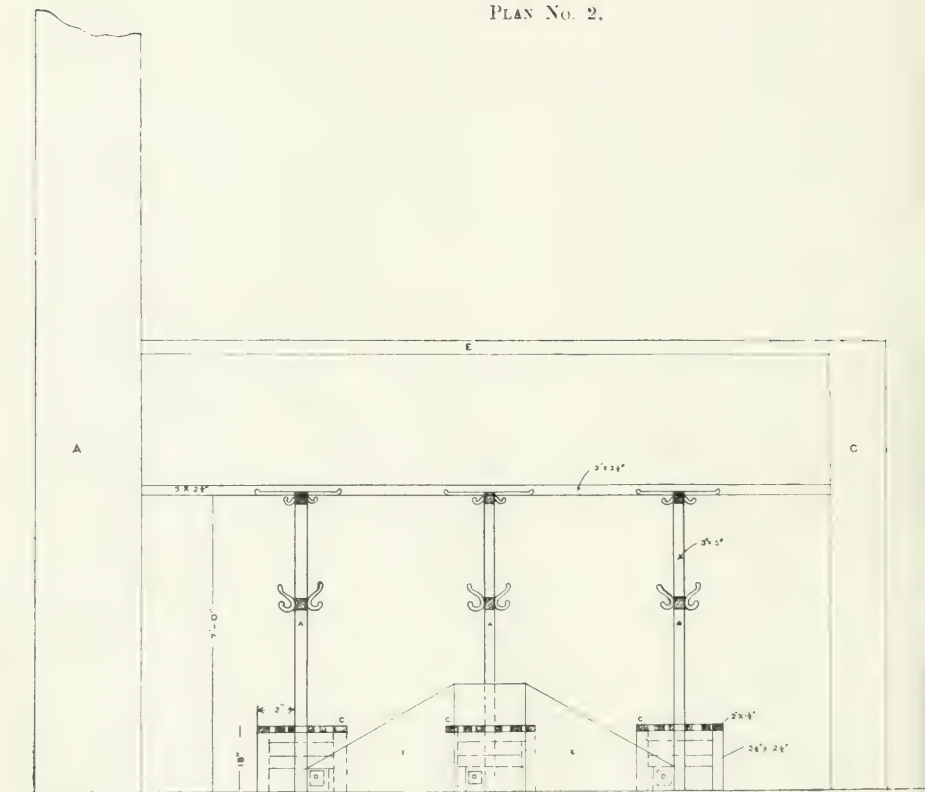
Experiments carried out in Bombay, with the assistance of Dr. S. N. Goré and Mr. Akula, showed that an exposure for 2 hours to a concentration of hydrocyanic acid gas starting with 100 parts in 100,000 and falling gradually to 30 parts per 100,000 was sufficient to kill bugs, fleas, lice and their eggs. Experiments with a very vigorous strain of lice, carried out at a later date, at the Lister Institute in London, with the assistance of Mr. Bacot, showed that, at a temperature of  $90^{\circ}$  F. and a concentration of 100 parts of HCN per 100,000 of air, all nits were killed when exposed for two hours, but a certain small proportion of adult insects escaped destruction. Very much longer exposures, even  $17\frac{1}{2}$  hours, failed to kill all active lice, while nits were invariably destroyed. The experiments carried out in London point to the fact that hydrocyanic acid gas can not be entirely relied upon to kill all lice, but, in view of the conflicting results obtained in Bombay, further experiments seem to be necessary. The lice used in London were breeding much more actively than those used in Bombay, and this fact may explain the divergent results obtained in the two places.

I take this opportunity of acknowledging the valuable assistance I have received from Dr. S. N. Goré in erecting and using this plant. I have also to thank Mr. Bacot of the Lister Institute for the assistance he rendered me in the experiments which were carried out in London referred to above.

PLAN No. 1.



PLAN No. 2.

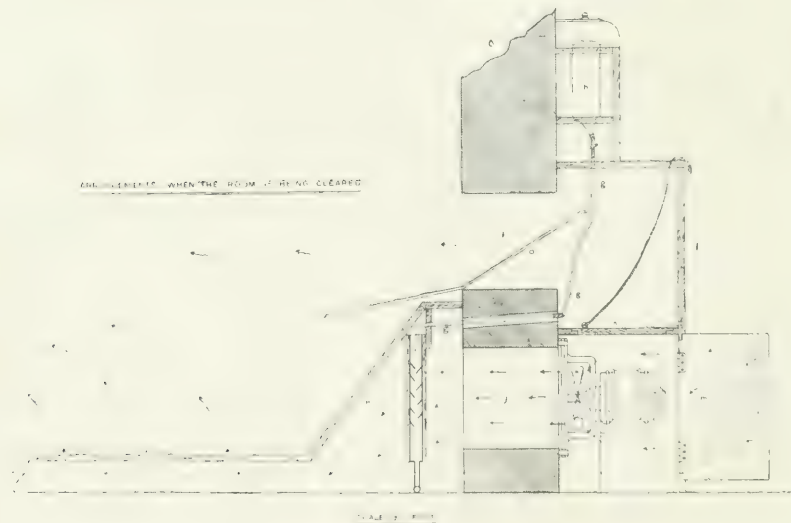
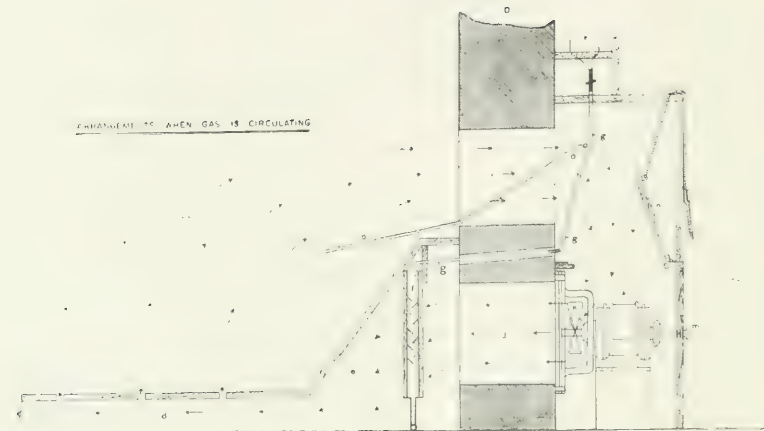


W. GLEN LISTON.—The Use of Hydrocyanic Acid Gas for Fumigation.

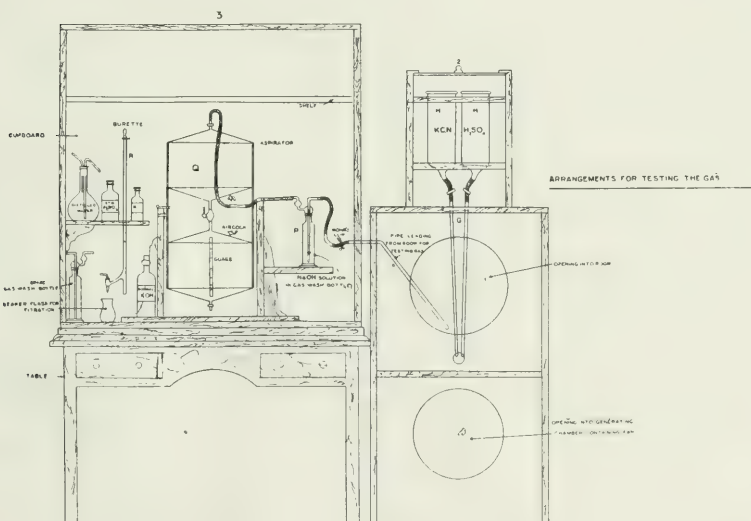
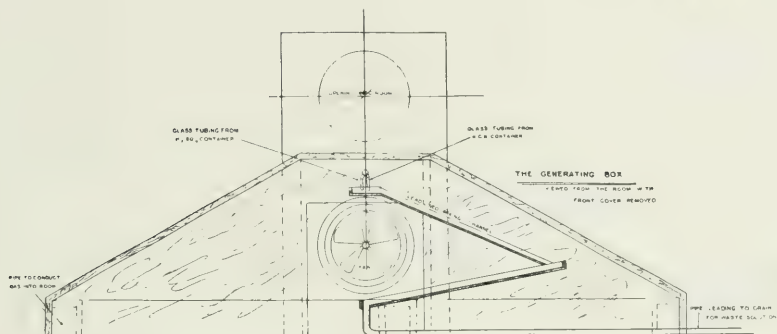




PLAN No. 4.



# PLAN No. 5.



SCALE 1/2" = 1 FOOT



# STANDARDIZATION OF DISINFECTANTS WITH SPECIAL REFERENCE TO THOSE USED IN THE CHEMICAL STERILIZATION OF WATER.

BY

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THE standardization of disinfectants is practically universally carried out by means of the Rideal-Walker method or one of its modifications. The method is the well known one of bactericidal effect in relation to time of action, and is carried out under defined conditions.

Many criticisms of the method have been forthcoming, chiefly concerned with the relation of the test to presence of organic matter. It has been suggested, for example, that, in order to obtain a result in which presence of organic matter was taken into account, sterilized pus, faeces, urine, etc., should be added.

It will assist in making clear the reasons for some suggestions we give in this paper for modification of the test (with special reference to water sterilizing substances) if we very briefly note what are the conditions now generally used to determine the coefficient for a disinfectant by the Rideal-Walker method. It is essential to any standard test that it should be capable of repetition by various workers under exactly the same conditions,

and in the Rideal-Walker method this is aimed at by the employment of the following standards. (Hewlett, 1914.)

(1) *Time*.—The time intervals at which the effect is noted are  $2\frac{1}{2}$ , 5,  $7\frac{1}{2}$ , 10,  $12\frac{1}{2}$ , and 15 minutes.

(2) *Organism*.—The test is usually made with a broth culture of *B. typhosus*, but other organisms may be employed (e.g., *B. coli communis*.)

(3) *Comparison with a standard disinfectant*.—The disinfectant used is 1-100 carbolic acid. The carbolic acid used in the Rideal-Walker test, as described in a War Office Memorandum (2), is:—'Carbolic acid prepared by distilling Grassar's pure acid and rejecting the first distillate and residue, the intermediate portion being reserved and tested by melting point for purity.' According to Hewlett, the carbolic acid used should have a melting point of not less than  $40^{\circ}\text{C}$ ., and should be kept in the form of a 5 per cent stock solution, standardized by the bromine method. In practice it may not be possible to obtain carbolic acid of these specifications. Samples of carbolic acid obtainable on the market are of variable character and the standardization of such differing samples is itself a matter of consideration.

In considering whether there was not some substance that might be used, having advantages over carbolic acid (especially as a standard for comparison with substances employed for the sterilization of water), we thought that iodine might be such a substance.

(1) In iodine we have an element very easily obtained in a pure form.

(2) The making of an accurate standard solution of iodine is a matter of direct weighing, and as iodine has no tendency to absorb water no special precautions have to be taken, as in the case of carbolic acid, in this respect.

(3) Since the substances most commonly used for the chemical sterilization of water are of the halogen group, a more homologous standard is likely to be obtained with iodine in suitable dilution than with carbolic acid.

For determining the coefficient of disinfectants used in the chemical sterilization of water (some of them proprietary preparations of unstated composition, but mostly containing chlorine), we have therefore employed iodine as the standard for comparison.

In addition to the use of iodine in the place of carbolic acid there are certain other modifications which, as will be seen later, are not merely

obligatory, but essential, if coefficients obtained for members of the halogen group are to have any real significance whatever.

(4) *Use of a broth of a standard composition.*—In order that the culture used may be of a standard organismal content, a broth is employed with the following composition :—

Lemco	..	..	..	20 grammes.
Peptone	..	..	..	20 "
Salt	..	..	..	10 "
Water	..	..	..	1000 c.c.

standardized to a reaction of + 10 (Eyre's scale).

We were fortunate in having a quantity of Witte's peptone with which to perform our experiments, but possible difficulty in obtaining a peptone suitable for the test is a matter to be taken into consideration, as the use of peptones of different characters is admitted to be liable to vitiate the result. (War Office Memorandum, 1919.)

Whatever may be the position of the Rideal-Walker coefficient in regard to its suitability as a final measure of the practical disinfecting value of a substance in the presence of organic matter, we consider that, in the first instance at least, it is necessary to compare the action of disinfectants simply upon the bacteria themselves. If the disturbing effect of extraneous organic matter is to be determined, it is preferable that an additional coefficient in regard to this point should be separately arrived at. In the Rideal-Walker technique, however, the action of the disinfectant is not ascertained in the absence of organic matter, for peptone and other substances contained in the broth are present. The necessity for the presence of such substances is evidently merely dependent on the method used for obtaining a standard bacterial suspension.

(5) *The addition of a fixed quantity of culture.*—The culture used is a 24-hour culture of *B. typhosus* or *B. coli* in the special broth. The quantity commonly added to a given amount of disinfectant dilution is 0.1 c.c. Along with the organisms there is obviously added a certain quantity of the constituents of the broth, amounting as regards organic matter to about 0.004 grammes.

It would seem evident that what is required in the test is a suspension of organisms without lemco or peptone. This may easily be obtained in 0.85 per cent salt solution from an agar slope. The suspension is standardized by means of an opacity scale such as that described by Brown (3) and Brown and Kirwan (4). This scale has the advantage moreover of expressing opacity in terms of weight of bacterial substance

in the desiccated condition. By substituting such a suspension for the broth culture it is possible to avoid the presence of organic matter other than bacterial substance itself in the test. In our earlier determinations suspensions were made in the usual way by the addition of salt solution to an agar slope culture. It was found, however, better to make a suspension from growth scraped off and shaken up in a separate tube of salt solution. The suspension we have used, except for special purposes, is one containing the equivalent of 1.0 mgm. dried bacterial substance to the cubic centimetre. Of this 0.1 c.c. is added to 3.0 c.c. of the disinfectant dilution.

(6) *Standard conditions of experiment.*—The standard procedure for the carrying out of the test has been employed by us in all respects except those noted.

*Coefficient of disinfecting power of iodine and chlorine.*

The only estimation of the coefficient of iodine we have been able to find is that given by Martindale and Westcott<sup>(5)</sup>, employing the *Lancet* modification of the Rideal-Walker test. This gives the effective limit of dilution of iodine as 1-50,000.

Our own determinations, using the unmodified Rideal-Walker method, shew, as a result of repeated determinations, a maximum effective dilution of 1-5,500. In performing the test it was noted that the tubes containing dilutions, when tested at the end of the experiment, gave a blue colour with starch solution *up to, but not beyond*, the particular point at which sterilization had been effected (1-5,500). Absorption of free iodine had evidently taken place in all the dilution tubes, and it was only at the point in the series where this absorption had failed to remove all the iodine that sterilization had occurred. The amount of absorption was found to be approximately that which occurred from the presence of the added broth constituents, so that the determination of a Rideal-Walker coefficient by this method appeared to mean nothing more than that so much organic matter (peptone and lemco) had required a particular quantity of iodine to be added before any was left free to act upon the organisms present.

The determinations made with a standardized suspension containing the equivalent of 1.0 mgm. dried bacterial substance to the cubic centimetre gave as the limit of dilution for effective sterilization by iodine 1-200,000. On using a suspension of one-tenth this bacterial content (0.1 mgm. per c.c.) the limit of sterilization was raised to 1-250,000.



The Rideal-Walker coefficient for 'available chlorine' as given by Martin (6) is 146-220, or dilutions of 1-14,600 and 1-22,000. Our own determinations with standardized suspension of 1.0 mgm. per cubic centimetre of salt solution shew sterilization up to 1-300,000.

In giving the limits of sterilizing effect we have purposely not entered time in the statement, since in the case of iodine and chlorine there is no difference shewn at the different intervals up to the 15 minutes at which the experiment terminates.

*Absorption of iodine and chlorine by bacterial substance.*

The amount of iodine absorbed by given quantities of bacterial substance in a given time was determined. The amount of such absorption was considerable and various estimations gave for 7½ minutes a value of from 0.08 to 0.11 grammes of iodine absorbed by one gramme of bacterial substance, depending upon the strength of the dilutions used and concentration of the suspension. In the dilute proportions used in the test there is a difficulty in making precise estimations owing to the fact that the presence of a certain amount of iodine is apparently required to give the blue test with starch in any given water and the exact relation of this minute quantity to sterilizing effect it has not been possible to determine.

Approximately the dilution at which sterilization will be found effective in the test when using a standardized bacterial suspension in salt solution was found to be given by the amount of iodine required to satisfy the absorbing power of bacterial substance present. To make this clearer we may reconstruct the conditions of the test as follows :—

Amount of bacterial substance added	..	..	0.0001 gramme.
Amount of iodine required (taking 0.1 mgm. iodine as absorbed by one gramme of bacterial substance).	..	..	0.00001 „
As this quantity would be contained in 3.0 c.c. the required concentration to cause sterilization will be one-third of this quantity per cubic centimetre, or 1-300,000 approximately.			

*Discussion.*

Hewlett (1) describes the Rideal-Walker method as 'an admirable one for determining the relative efficiencies of disinfectants on *naked* organisms in the *absence* of organic matter.' It will be seen that, on the contrary, in the method as frequently employed, there is a very important consideration, *viz.*, the existence in the experimental tubes of substances, peptone, lemco, etc., having possible combining powers with the substances to be tested and deflecting the action of the disinfectant from the

organism to such an extent, in the case of the halogen group, as largely or completely to vitiate the result.

In the modification we have employed it appears to us that we have a more satisfactory coefficient which does actually indicate the effect of the disinfectant on a definite quantity of naked organisms in the absence of organic matter.

But in reporting upon the value of a disinfectant there are quite a number of considerations that ought to be taken into account. It does not follow necessarily that a substance giving the highest coefficient in regard to sterilizing power upon naked organisms is the best. Other considerations may come in, such as efficacy of the material in the presence of organic matter, relative price of the material in effective dilution, stability, etc. In the war the question of portability naturally had great weight. Bleaching powder, for example, had the great advantage of portability but the great disadvantage of rapid deterioration and of a corrosive effect upon metal receptacles. A solution of bleaching powder (hypochlorite) standardised by titration satisfied the condition of sufficient stability for the purposes required, but was bulky for transport purposes. Again, we have found that a water disinfectant sent to the Central Research Institute for test contained a mercury salt, so that the element of poisonous character should also be indicated in the case of disinfectants used for such purposes as water sterilization. To sum up, the best type of report upon a disinfectant is one giving the determination of a number of coefficients :

- (1) Coefficient of efficacy. Determined on bacterial suspension in salt solution.
- (2) Coefficient of availability. Determined in relation to organic matter.
- (3) Coefficients of stability, price, portability, poisonous character, etc.

In regard to the halogen group with which we are now mainly concerned, it is possible by our method to give quite useful figures in regard to any proprietary substance claiming special value as a water sterilizing preparation.

#### CONCLUSIONS.

(1) The suspension of organisms used in testing disinfectants should be one of naked organisms in a menstruum containing no organic matter ; special coefficients should be devised for the evaluation of the effect of organic matter.

(2) The quantity of organisms contained in unit volume of the suspension is a necessary datum in the evaluation of a coefficient of efficacy.

(3) Iodine is a better indicator to use (at all events for the testing of water sterilizing substances) than the commonly used carbolic acid ; it has several advantages over carbolic acid which might render it preferable to carbolic acid in other cases than that mentioned.

(4) Coefficients of stability, price, portability, poisonous character, etc., are required to complete the description of a disinfectant.

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# THE ETIOLOGY OF SPRUE.\*

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LATE CAPTAIN, R.A.M.C.

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## I. DEFINITION OF SPRUE.

Sprue, or psilosis, is thus described by Sir Patrick Manson <sup>(26)</sup> :

'An insidious, chronic, remitting inflammation of the whole or part of

\* Being a thesis for the degree of D. M., Oxford University, 1919.

*Note.*—This paper is published as a review of the subject. It gives a resumé of what is known and presents suggestions as to lines of future research.—Editorial Committee.

the mucous membrane of the alimentary canal, occurring principally in Europeans who are residing or have resided in tropical or subtropical climates. It is characterized by irregularly alternating periods of exacerbation and comparative quiescence ; a peculiar, inflamed, superficially ulcerated, exceedingly sensitive condition of the mucous membrane of the tongue and mouth ; great wasting and anæmia ; pale, copious, and often loose, frequent, and frothy fermenting stools ; very generally by more or less diarrhœa ; and also by a marked tendency to relapse. Sprue may either be primary, or it may supervene on or complicate other affections of the alimentary canal. Unless properly treated it is usually fatal.'

## II. SCOPE OF PAPER.

The above definition is purely clinical. The cardinal signs of the disease are seen to be four, namely (in the succinct words of an American author), ' a sore and beefy tongue, a gaseous bowel, a foamy light-coloured and abundant diarrhœa, and a small liver '(1). They are indeed sufficiently well-marked, and differential diagnosis seldom presents less difficulty than in a case of sprue. But clinical definitions, even from the clinical standpoint, are seldom satisfactory, for they imply empirical therapy. Many cases, or even most cases, are no doubt adequately recognized and effectively treated by these means ; but, as in every other disease, abnormal, early and doubtful examples will occur, which call for more exact methods. On scientific grounds a clinical definition is scarcely better than no definition at all. If its tendency is to premature conclusions and prejudiced judgment it is even worse. Yet if an evil, it is a necessary evil : in all medicine the inevitable order of procedure, both historically and practically, has been first treatment, then diagnosis, and lastly causation. The progress of accurate scientific knowledge of disease is constantly delayed by this practical necessity for putting the cart before the horse.

In the case of diseases of obscure origin, chronic course, and rare occurrence, the difficulty is the greater, owing to fewer opportunities for observation of the early stages, and for autopsy. It may happen that the early stage of such a disease receives another name, and is regarded as a distinct malady ; on the other hand, it may be, causes which are merely predisposing are regarded as exciting. The problem for the investigator in such cases is indeed to pierce through this disguise, and recognize essential elements from those which are variable, accidental, or secondary. In relation to sprue this point will recur later.

Again, a perfectly definite morbid condition may be determined by more than one cause. The essential lesion has then to be distinguished from the variable preliminaries. Etiology is concerned, not with the train of circumstances, often trivial, leading up to the development of a state of disease, but with the particular link in that chain or train, which differentiates the disease from all others. Tuberculosis, for instance, may be the result of insanitation, or of a hereditary taint, but the essential etiology is infection with the tubercle bacillus. Etiology and pathology in fact are hand in hand; and till the nature of a disease is accurately known its causation also is likely to be misunderstood.

The scope of the following thesis will be a contribution to the etiology of sprue, a disease which has suffered greatly from such misunderstanding. To this end a brief statement of the various theories of sprue will precede a more detailed consideration of its truly remarkable characteristics, studied in their relation to etiology. Finally, an attempt will be made to draw inferences from a summary of these data, and to form an opinion as to the cause of the disease.

### III. THEORIES OF ETIOLOGY.

Of the suggested causes of sprue, as enumerated in Castellani and Chalmers' text-book, four may be dismissed very briefly.

(i) Sprue is a complex syndrome, but no specific disease at all. This view can hardly appear probable to anyone familiar with the remarkable and characteristic appearance of a typical case. Its causes are doubtless obscure, and the contributing tendencies many, but the resulting disease is certainly a definite pathological entity.

(ii) Sprue is a food disease. Tinned and spiced foods, high living, the abuse of alcohol, combined with the effects of a sedentary life, have been adduced as the causes of sprue, as of 'tropical liver.' No doubt these factors play a part, and an important part, in the development of many cases; but there are others in which they may definitely be excluded. The greater incidence of sprue in women, for instance, is unaccountable on this theory. I have seen a case of a European who developed sprue after living only nine months in India and being dieted carefully throughout on account of the history of a duodenal ulcer. Nor can these factors be responsible for producing the disease, for instance, among the low-class natives of Ceylon.

Sprue has also been attributed to diet-deficiency; notably in 1851 by Grant, who considered that the mouth-lesions indicated the scorbutic

taint'; for scurvy, he says, 'is by no means always dependent on the want of vegetables.' Manson <sup>(26)</sup> suggests the possibility of a 'physiological famine,' on the analogy of the many varieties of diarrhœa and dysentery developing in famine camps. Buchanan<sup>(7)</sup>, who states that sprue is common among the natives of Bengal, observes that it is very similar to famine dysentery, and to the chronic dysentery of Indian gaols. The danger of scorbutic complications in sprue cases where the absorption of food-elements is long restricted, is apparent: but the wide climatic distribution of sprue, and its prevalent age and race incidence, are difficult to reconcile with diet-deficiency. There is some slight similarity between sprue and pellagra, a known deficiency disease, and the two conditions have been confused <sup>(9)</sup>, though they have not actually much in common <sup>(23)</sup>.

(iii) Sprue is a helminthiasis. Strongyloides infection has been found in a number of sprue cases. It is however far from constant, and is equally common in tropical pathological conditions of all kinds.

Two of the suggested causes remain: namely (iv) infection, and (v) a physiological exhaustion of some part of the digestive mechanism. Under the last must be included (vi) individual idiosyncrasy, an unsatisfactory term, yet expressive of a well recognized physiological fact, which might fairly be held to account for some of the peculiarities of sprue-incidence among populations exposed to similar external conditions.

(iv) Sprue is an infection. This is the commonest view. Indeed, it represents at the present time the considered opinion of almost all authors. Beneath this appearance of unanimity, however, there is a lack of real agreement, and the arguments in favour of one type of infection are opposed to those in favour of another. Regarded therefore as the sole cause of sprue, the three theories which are included under this head are mutually exclusive: in so far however as they may be merely occasional and accessory causes, all of them may play a part.

(a) The infective agent is a fungus. This is the view first formed by Dantec. Bahr <sup>(4)</sup> considers the fungus to be *Monilium Albicans*, the organism responsible for thrush among European children: according to Ashford, it is an allied variety. The presence of such fungi in sprue is generally recognized: they are usually recoverable from the stool, and occasionally from the mouth-ulcers: to their action the fermentative changes in the contents of the bowel in sprue are principally due.

Castellani and Low <sup>(15)</sup>, however, have shown that the presence of these fungi is only incidental; and that while they can be destroyed,



or their activity at least checked by the administration of alkalies, the disease progresses. Mycotic infection, moreover, is very common in the tropics, in normal subjects. Ashford <sup>(2)</sup> states that the fungi which he has isolated in 100 per cent of cases from the tongue and stools, are pathogenic to small animals, and are to be found also in bad bread, to the eating of which he attributes the disease. Sprue, he says, is only the last stage of common fermentative diarrhoea. This belief he considers to be supported by the efficacy of a milk diet. 'The very fact that some cases are permanently cured by these diets alone, without any medicine whatsoever, makes me extremely suspicious of the asseveration of Chalmers and Castellani that sprue is not likely to be a mycosis, but is probably a protozoal disease.' The striking fact to which he alludes might however, perhaps be considered almost equally strongly opposed to the mycotic theory.

(b) The infective agent is a pyogenic micro-organism. Rogers <sup>(30)</sup> has had considerable success with the use of vaccines of streptococci cultivated from the mouth. Castellani <sup>(13)</sup> similarly has cured cases with vaccines prepared from bacilli of the Flexner group recovered from the stool, and agglutinating with auto-serum. Such cases however he considers to be exceptional, though they resemble sprue very closely. He calls them 'pseudo-sprue.' Neither does Rogers seem to regard streptococcal infection as the sole cause of the disease. 'It would not be surprising,' he says, 'if the digestive derangements which not infrequently develop into sprue in tropical climates, should sometimes be followed by infection of the intestinal canal with the pathogenic oral streptococci so often found, in almost pure culture, in pyorrhoea and other inflammatory and ulcerative conditions of the mouth, and which form such an important part in the clinical picture of sprue itself. Rogers and Castellani, in fact, would seem to agree that the bacterial infective element in sprue, even if fully demonstrated, and even if amenable to specific bacteriological therapeutics, with complete cure of the disease, is secondary and a complication, rather than the true and essential pathological condition.

(c) The infective agent is an unknown parasite, probably a protozoon. This opinion is held by the greatest number of modern authors—Castellani and Chalmers <sup>(11)</sup>, Manson <sup>(25)</sup>, Carnegie Brown <sup>(16)</sup>, Thin <sup>(36)</sup>, and Begg <sup>(5)</sup>. Ashford <sup>(1)</sup> and Bahr <sup>(3, 4)</sup>, as noted above, are opposed to it, and there is no direct evidence in its favour. Specific organisms do not cause fermentation, and they do not cause stomatitis, for which, as

already mentioned, other causes have been found ; and, to assume that the underlying pathological condition responsible for these sequelæ, is due to the unknown activity of an unknown parasite, would seem somewhat gratuitous. In exceptional cases, associated with stomatitis due to pyorrhœa, Schmitter (<sup>31</sup>), and also Rogers, have obtained benefit or cure by administration of emetine, together with ordinary dietetic treatment.

(v) Sprue is due primarily to exhaustion of some part of the digestive function. Sprue, in fact, is a climatic disease. No disease, strictly speaking, is, nor can be, climatic ; but climate may well influence, favourably or unfavourably, physiological functions, and stimulate or paralyse their activity. On this theory infections of various kinds may help to bring about physiological failure ; more likely still, infection may be its sequel ; but the essential characters of the disease depend upon the cessation of one or more of the digestive secretions. The climatic associations of sprue will be mentioned later, and the reasons for supposing that this is the correct theory of sprue ; but, speaking generally, the limitation of the incidence of the disease to regions with a hot, moist climate where physiological vitality is at a minimum, together with the marked influence of a change of climate in hastening cure, are sufficient reason for maintaining this provisionally as among the possible causes of the disease.

#### IV. FEATURES OF THE DISEASE, WITH THEIR BEARINGS UPON ITS ETIOLOGY.

Towards a decision between these theories of the origin of sprue, the many peculiarities which place this affection in a quite unique position among tropical diseases should, one would think, be of sufficient assistance. The facts group themselves naturally under the usual headings of (i) Incidence, (ii) Symptoms and Signs, (iii) Affinities with other diseases and Differential Diagnosis, (iv) Morbid Anatomy, (v) Course and Prognosis, and (vi) Treatment.

(i) (a) Race. Sprue is essentially a white man's disease. Among the teeming native populations of the countries where it is endemic, cases have, it is true, been observed. The stools of the vegetarian Asiatic approximate normally to the sprue-type, and the incidence of sprue may therefore have been in some cases overlooked. 'Ceylon sore-mouth' is common among the natives of Ceylon. The Indian populations both of Bombay and Bengal, belonging to many different

ances, are said to suffer not infrequently from sprue. Van den Burg found many native cases in Sumatra. Yet, on the whole, considering the relative numerical proportion of the different race-populations, sprue must certainly be regarded as excessively less common among Asiatics than in Europeans. Nor is this relative native immunity purely local; thus Canton is free from endemic sprue, yet the Cantonese coolie immigrating into the Malay States escapes whilst the immigrant European is attacked.

Again, among the Europeans in a sprue district, the greatest incidence is amongst those born in the country, or who have long been resident in it. There is no acquired immunity, but rather the reverse. It is the newcomer who is comparatively immune. Thus out of 41 cases among Europeans in Calcutta, Rogers (<sup>20</sup>) finds 26 to have been born in India. Sprue is thus unique among tropical diseases in attacking principally the acclimatized foreigner, and in usually sparing both the native and the newly-arrived European.

(b) Age. Sprue, as has been frequently observed, distinguishes neither between rich and poor, nor between the robust and the weakly, nor, in adults, between the young and the old. The only unaffected members of the European community are the young children. Rogers gives the following age-incidence among 47 cases in Calcutta:—

	Up to 10 years.	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60	Over 60	Total.
Males . . . . .		3	9	5	8	4	1	30
Females . . . . .		5	2	9	1	—	—	17
Total . . . . .		8	11	14	9	4	1	47

These figures are roughly proportional to the total population in each decade, and to the relative numbers of the two sexes. Others have stated that the percentage among women is slightly the greater: it is certainly not less. The figures are chiefly remarkable for the immunity they show among children. This is a rare feature in tropical medicine, particularly in the intestinal group of diseases. Though children are as a rule well cared for in the East, and thus protected from many pathogenic influences, they are extremely prone to most digestive disorders, and suffer severely from them. It is noteworthy that *Monilium Albicans*.

supposed by Bahr to be the cause of sprue, is the fungus of thrush, a characteristic disease of children.

(c) *Geographical Distribution.* Sprue occurs widely throughout the tropical belt, especially in China (Southern), Indo-China, Java, India and Ceylon. It is reported also in the Southern States of the U. S. A., the West Indies and Porto Rico, North China, Japan and elsewhere. Of a hundred cases reported by Begg (<sup>5</sup>), as having been seen by him in England, 33 came from India, 33 from China, 10 from Ceylon, 9 from Malay, 2 each from Japan and the Congo, and 1 each from Morocco, the Gold Coast, Siam, and Java. In view of the remarkable latency sometimes shown in this disease (one case, reported by Thin, having developed symptoms 17 years after leaving the East), some of these cases were possibly imported. The geographical range of the disease however is certainly extremely wide: it is associated with a certain type of climate. 'Apparently sprue is most prevalent in those tropical countries in which prolonged high temperature is combined with a moist atmosphere.' (Manson.) Given such climatic conditions the presence of sprue may confidently be expected.

There are however certain anomalies in this distribution. Sprue may occur after very brief exposures in an endemic area: Roux mentions the case of a patient who had stayed in a tropical port only for the coaling of his ship. The incidence in different districts apparently identical in climate is very variable. Thus it is common in Malay, rare in Central America; common in Shanghai, rare in Japan. These facts, however, are open to the criticism that sprue is not always effectively diagnosed. Its mortality moreover has been probably underestimated, from the attribution of death in sprue-patients to some terminal complication. The only legitimate inference from the known facts of sprue distribution would seem to be that sprue occurs wherever the atmosphere is constantly hot and moist, and rarely develops elsewhere.

The peculiarities of the incidence of sprue are therefore that while it may occur at all ages, in all races, and in many countries, it is found chiefly (a) in Europeans, especially those long resident in the East; (b) in adults; and (c) in all hot, moist climates. It is difficult to reconcile these characteristics with a specific infection, or indeed with an infective process of any kind. How does an endemic infection come to be so widely spread? Why should the native be spared? Why should children be spared? If natives have developed relative immunity, why should Europeans not only fail to acquire immunity, but rather grow

increasingly susceptible? Why should Orientals, living in countries such as Hong-Kong where there is little or no sprue, be immune when they migrate to sprue-districts? How should a disease so rare as sprue appear in countries so far apart as, for instance, Ceylon and Porto Rico? Why is not the infection spread by sprue-patients returning to Europe? In connexion with the last point it should be mentioned that alleged instances of infection have been recorded (Carnegie Brown); but they are admittedly rare, and the supposedly infected persons have always themselves been exposed to an environment where sprue is endemic.

If, on the contrary, the essential defect of sprue be not infective, but physiological, the facts are more readily explicable. Europeans whose diet is excessively rich and indigestible, and whose mode of life is strenuous and often injudicious, would naturally be more prone to an exhaustion of the digestive mechanism than the more sedentary native who lives simply. The predisposing influence of the strenuous life and irregular meals is attested by Rogers, who states that 10 out of his 30 male patients were employed on the railway. Prolonged exposure to exhausting influences would naturally intensify the liability to attack, and the resident of long standing would therefore be the most likely to contract the disease; while the racial acclimatization of centuries would naturally induce a condition of physiological compensation, leading to a relative immunity of the native, whether his particular home lay in an endemic area or not. The climate where sprue is commonest is that most unfavourable to the maintenance of glandular activity, though its first effect is no doubt stimulating. Prodrómata indicative of hepatic hyperactivity are often noted in the history of sprue patients. Children, again, would naturally be immune to a disease caused by exhausted vitality. There are occasional anomalies, it is true, both in the distribution and in the incidence of sprue, and it is often difficult to explain them. The chain of cause and effect in individual cases is admittedly obscure; but the broad facts would not seem to be unintelligible, if the essential cause of the disease is sought, not in a specific infection, but in a faulty or inadequate reaction of the individual to his environment.

#### (ii) Symptoms and Signs.

(a) 'A foamy, light-coloured and abundant diarrhœa.' This description, though accurate, scarcely emphasizes sufficiently the remarkable appearance of the sprue stool. Three stages are noted in its appearance during the progress of the disease. First, preliminary to all other symptoms, there is a loose, biliary diarrhœa, with highly-coloured watery

offensive stools. This form of preliminary diarrhœa has been noted by Manson and most authors ; it is absent in such cases as supervene upon hill-diarrhœa or chronic dysentery. Secondly, in active sprue, the stool is buff-coloured, creamy in consistency, and full of air-bubbles. It may not be passed very frequently and the actual number of stools passed is not a reliable criterion of the progress of the disease. It is passed most often in the night or early morning, with explosive discharge of gas and temporary relief of the abdominal discomfort, from which at this stage the patients nearly always complain. The third stage begins, in a typical case, within a week or less of the commencement of a strict milk diet. The stools now are fully formed, and passed without flatulence once or twice in the day. They are still pale in colour, greasy, and very bulky. The enormous size of the motion is usually remarked by the patient, and is indeed very striking. In this stage improvement has set in and an increase in weight begun. There are no acute symptoms. In contrast with his previous misery the patient feels extremely well. The stools remain pale and bulky for a considerable time ; in a successful case the normal appearance gradually returns. Till it has completely done so, the patient is in danger, and irregularity of diet is liable to bring on a relapse. This tendency is indeed never absent in a man who has had sprue, but sprue which relapses after the stools have once become normal should probably be regarded rather as a fresh attack of the disease than as a true relapse. The cure is complete when the digestive function has returned to normal, and of this event the character of the stool affords the most reliable indication.

Each of these stages in the metamorphosis of the sprue-stool ought to throw light on the pathology. The first indicates an irritable, over-stimulated digestive function, the second a failure of absorption and fermentative decomposition by gas-forming organisms, the third a simple failure of digestion or absorption of fat.

The second stage, when the disease is most active, and an acid, fermenting, frothy diarrhœa conspicuous, is naturally that upon which most attention has been fixed ; and the obvious role of organisms in contributing to it has led to the general belief in the infectivity of sprue. But the first and third stages, whose existence has scarcely been more than noticed, are surely, from the standpoint of etiology, no less remarkable. Sprue may or may not be an infection, but the sprue-stool in its early stages and during the period of so-called convalescence, is manifestly due to a defect in the mechanism of digestion. The true cause of



sprue is not the cause of the intestinal putrefaction which leads to the flatulence and diarrhœa, for the sprue persists after this process has been arrested, and may be said in many instances to have begun before it sets in. The character of the stool in sprue lends no support to the belief that the disease is essentially an infection, for the evolution of this symptom during the normal course of a successful case indicates merely a disturbance of digestion, commencing with hyper-activity and continuing with failure, from which recovery slowly follows after its consequences have been arrested. The fact that sprue is usually preceded by acute bilious diarrhœa, if not by some other variety of intestinal upset, points also to the probability that in the later stages there is present a state of exhaustion of function to which a variety of causes may have predisposed. A disturbance of physiological equilibrium may well be the end-result of such variety of factors; but the incidence of specific infections is not usually of this kind.

(b) 'A gaseous bowel.' Extreme flatulence and the resulting abdominal discomfort are the principal complaints of most sprue patients. The abdomen on palpation is said to be 'doughy'; it feels like palpating an air-cushion. The frothiness of the stool has already been noticed. Fermentation is evidently proceeding very actively; but it is not an integral feature of the disease, being present in only one of its stages; and though it is a troublesome symptom, and the decomposition which it implies must hasten the cachexia of cases unsuccessfully treated, its presence throws little light on the subject of etiology.

(c) 'A sore and beefy tongue.' When sprue has reached an advanced stage, extensive changes have usually taken place in the appearance of the tongue. It will be found raw, red, and cracked. It is hyper-sensitive; the patient is intolerant of strongly-flavoured food. The mere mechanical act of swallowing, or even of speaking, may be acutely painful. In earlier stages there may be partial denudation of the mucous membrane round the edges and at the tip, or painful ulcers on the lips and tonsils: the lesions vary in size from large sores with sloughing base to minute foci of ulceration or patches of aphthous stomatitis; or merely subjective hyper-sensitiveness. Tongue symptoms of this kind have frequently been described as an early indication of sprue, even as the earliest. They occur however irregularly, being especially common, early, and prominent in cases of sprue contracted in Ceylon, and comparatively rare or trivial in the sprue of India. If the disease progresses the mouth lesions also usually progress; sometimes however their severity



seems to alternate with that of the diarrhoea. They also show remarkable intermissions. For weeks they may disappear altogether, and then recur; the tendency being to become gradually more and more severe, and more and more extensive; though in the last stages of cachexia the amount of discomfort seems somewhat to pass away. When treatment is successful, the relief of the mouth-symptoms is usually among the first signs of improvement: and indeed they are usually no longer evident thereafter, though other stigmata of the disease may long persist. The tongue and mouth symptoms of sprue therefore, though striking and characteristic, are not an essential feature. According to Rogers (<sup>29</sup>), they were completely absent in 8 out of a series of 43 cases; in many more they play only a trivial and transitory part.

From the standpoint of etiology it would be legitimate to say that sprue is characterized by a tendency to develop sores in the tongue and mouth. The actual extent of the lesions is variable and, in this sense, unimportant. The distinction is, I think, not unnecessary; for if the disease be due to a specific infection, definite lesions would be anticipated; whereas if its causes were rather physiological, effects would more likely be manifested as variable in degree, but constant in tendency.

From oral ulcers Rogers (<sup>30</sup>) has obtained a streptococcal vaccine whose use he has found very beneficial: but, as he points out, this does not carry etiological proof of the cause of the disease, for the treatment of complications may be as effective as treatment of the essential lesion: all treatment of sprue, as in fact of most diseases, being based on this principle, that is to say, being symptomatic only. The importance to etiology of these mouth-symptoms consists in the fact that they may be the only lesion present. The suggestion has been made that sprue is essentially a mycotic infection of the alimentary tract, giving rise to stomatitis, to gastritis, or to diarrhoea, according as the infection involves the mouth, the stomach, or the bowel. Against this view may be noted the facts that under appropriate treatment of an early case the mouth-symptoms clear up as a rule very rapidly, while the disease persists; that stomatitis is extremely common in the tropics; and that relapses of sore-mouth in sprue are the common sequelæ of indiscretion in diet, which might perhaps disorganize a recuperating but enfeebled power of resistance, but could hardly be thought to determine the re-infection of the mouth by specific organisms.

(d) 'A small liver.' This is almost a constant sign in untreated sprue. The diminution of the liver-dulness is very marked, and out of all proportion to the degree of hepatic atrophy discovered post-mortem. It also fluctuates markedly. Begg, an enthusiastic advocate of santonin treatment, claims that the liver dulness may return to normal in 24 hours after use of santonin, or even ammonium chloride. From this it might appear that this liver-shrinkage was more apparent than real, due perhaps to flatulent distension of the bowel. The actual diminution in the size of the liver is however shown on the X-ray screen, where the right dome of the diaphragm is found to be markedly flattened in sprue-cases. Fluctuations of this kind and degree are explained without great difficulty as a phenomenon of physiological exhaustion or disuse; but they would not seem likely as manifestations of a specific infection.

(iii) Affinity with other diseases. Apart from some confusion with pellagra and chronic dysentery the clinical difficulties of differential diagnosis in sprue are not as a rule great: the picture is usually unmistakable. Until the pathology of the disease is placed on a sure basis, however, its recognition when in a typical form must remain uncertain. At present diagnostic difficulty occurs when, as sometimes happens, the cardinal signs are not all present. The mouth symptoms, for instance, may be absent, especially in cases from India: or, again, they may be the only symptom, and, so far as one can see, the only disability. Such cases have sometimes been given different names, such as diarrhoea alba and Ceylon sore-mouth; or they have been grouped together as 'incomplete sprue.' A large number of conditions, which may or may not be sprue, have received local names, without being completely investigated. There are two however, namely, Ceylon sore-mouth and Indian hill-diarrhoea, which are well-recognized entities; and since these conditions are not generally familiar a short description of them will be given.

(a) Hill-diarrhoea. This is an acute morning diarrhoea, affecting Europeans at hill stations in the Tropics, especially in India, in the monsoon or rainy season, especially at its beginning. During this season hill-diarrhoea is endemic: occasionally, as in the famous 'Simla trots' of 1880, it assumes violent epidemic form. It attacks mostly newcomers to the East: but some subjects appear to be susceptible, and in these it will recur regularly each year, within a few days or even hours of arrival in the Hills. It does not affect children. The diarrhoea, confined usually to the mornings, is copious, pale, acid, and frothy, like whitewash. Dyspepsia and flatulent distension are usual and troublesome. There

is a furred tongue, but no soreness about the mouth-parts, nor ulceration. There is no diminution of liver-dulness. The standard treatment is by milk diet, with pepsin and perchloride of mercury. Such treatment is usually successful, or indeed the disease may sometimes pass off spontaneously. In other cases a return to the plains is necessary, and occasionally, even in spite of this, the cases will pass on into chronic sprue.

The clinical resemblance between the diarrhœa of sprue, and hill-diarrhœa, is almost exact, and they were once considered to be the same disease. More recently they have usually been regarded as distinct, emphasis being laid upon the acute onset, the absence of mouth-symptoms, the normal size of the liver, and the completeness and rapidity of cure, which characterize an attack of hill-diarrhœa. These differences however can hardly be considered crucial, nor to be more than those between an acute and a chronic form of the same disease. Moreover, as already mentioned, a number of cases of hill-diarrhœa pass into unquestionable sprue; and all stages exist between the comparatively mild 'diarrhœa alba' (which, but for the fact that it may develop in the plains, is clinically indistinguishable from hill-diarrhœa), and the most fatal form of sprue. The following case illustrates such a transitional form of the disease:—

Lieutenant W. S., aged 32, arrived in India in 1917, and was stationed in the plains at the beginning of the cold weather, and enjoyed good health, living abstemiously and taking regular exercise. In April 1918 he went to Mount Abu (4,000 ft.). Diarrhœa started almost at once, and the stools, which were at first bilious, soon became pale in colour, bulky, and frothy. He returned to Bombay in August, and the stools were then typical of sprue. The tongue was furred, and there had been no ulceration nor sensitiveness of the tongue and mouth. The abdomen was distended and doughy. There had been a rapid loss of weight. The liver-dulness was notably reduced, measuring scarcely 2" in the nipple line.

For a week he was treated with pepsin and perchloride of mercury, and became steadily worse. The diagnosis was then definitely changed to 'sprue,' and a strict milk-diet was commenced, with chalk and pancreatine and liver-soup. Improvement commenced almost immediately, flatulence and diarrhœa ceasing and weight being quickly put on. For the following month however the stools remained bulky and pale, and one or two relapses occurred after slight changes in the dietary. He gradually recovered.

This case clearly was one of sprue, without mouth-symptoms ; but in its origin it was as clearly a hill-diarrhœa. A distinction between the one disease and the other was never possible.

(b) Ceylon sore-mouth. This condition has been carefully studied by Bahr <sup>(4)</sup>. He states that it is especially common in Ceylon among the natives, and is occasionally though rarely associated with other symptoms of sprue. Possibly it occurs also in the natives of other tropical countries as yet insufficiently studied. It presents all the characters of the sprue-tongue. That is to say, in the earliest stages there is no obvious lesion, but only subjective sensitiveness, and intolerance of the spicy foods of which these races normally are inordinately fond. Later aphthous patches appear, especially at the edges, opposite the teeth ; chronic ulceration, intermittently active and indolent ; and finally widespread denudation of the epithelium and deep painful cracks. Sore-mouth without other sprue stigmata is rare among Europeans, showing thereby an incidence exactly opposite to that of ordinary sprue. Moreover it apparently occurs principally among the lowest ranks of society, and in the jail-population, and differs somewhat from the mouth of sprue in tending to exhibit a steadily progressive course.

Though Ceylon sore-mouth and sprue are found thus independently, and in different sections of the community, and with a different geographical distribution, the clinical resemblance between the oral lesions in the two conditions is very great. There can scarcely be any doubt that the cause for both is the same. The nature of this cause however remains obscure ; Ceylon sore-mouth is by no means certainly a specific infection though the infective element of course is always present. It would seem on the contrary to depend upon a state of lowered physiological resistance, affecting in the one case the antiseptic resistance of the buccal epithelium, and in the other both this and the vitality of the digestive function.

(iv) Morbid Anatomy. The pathological changes that have been described in the post-mortem room on cases dead of sprue have been rather contradictory. In a wasting disease of the digestive organs the findings, as only to be expected, are complicated by secondary and terminal changes : no record, unfortunately, exists of any autopsy of a spruecase after accidental death in an early stage. In addition to intense anæmia, emaciation, and desiccation of all the tissues, a widespread ulceration of mucous membrane throughout the whole of the intestinal tract has been exhaustively described by Bertrand and Fontan, whose results are quoted

by most recent writers ; but they do not throw much light on the nature of the primary pathological process. In one autopsy made by Wethered and quoted by Begg (<sup>5</sup>), the ulcerative process in the intestine appeared definitely to have originated in the ileum. The liver is usually found to be either congested or atrophic, the pancreas pale and indurated ; but these changes are not constant, and both organs have been found repeatedly to be both macroscopically and histologically normal. The negative value of this last fact is, from the etiological standpoint, the most important fruit of post-mortem work on sprue ; for if sprue may exist, and end fatally, without any necessary organic lesion in either of these organs, in spite of the evident failure of function of one or other or both, a physiological causation of the disease would seem clearly indicated. It is difficult to conceive of an infective disorder, disturbing digestion to the point of causing death from inanition, and yet terminating fatally without having caused any recognizable abnormality in the structure of those organs whose function it has destroyed.

(v) Course. The onset of sprue in most cases is exceedingly insidious. Chronic intermittent diarrhœa may long persist without attracting especial attention, and the flatulence which accompanies it is often put down lightly to ' indigestion.' The disease may eventually be discovered accidentally or owing to the supervention of mouth-symptoms. The preliminary diarrhœa, as already mentioned, is often bilious in character, and quickly forgotten by the patient : hence it is possible that these mouth lesions are not always so definitely a prodromal symptom as some authors have described. Sprue may also follow on dysentery or hill-diarrhœa or women's diseases or child-bearing—in fact, ' any depressing influence, especially if it is combined with intestinal irritation ' (Manson). Further it may exhibit considerable latency, the symptoms first developing months or years after the Tropics have been left behind.

In its course, if untreated, sprue is similarly chronic. Relapses are frequent, alternating with periods of quiescence and even improvement. Though the tendency is to become progressively worse in each relapse, the end is long postponed, to the extreme limit of emaciation ; and death, when it comes, is generally from intercurrent disease. In all but the last stages the disease may usually be arrested and frequently cured by strict dieting, or a change of climate ; but even in those most successfully treated relapses are liable to occur for months after the cessation of all symptoms, following as a rule on exposure to chill or indiscretions in diet. Many patients, however, have been permanently and completely cured

and return to the Tropics to lead their former normal life, without ill-effects. Sprue is a disease involving widespread destruction of the mucous membrane of the alimentary canal; the prospects of a cure depend probably upon the limits of its regenerative power. When the process has been too virulent, or is too advanced, or when the vitality of the subject is too low, from old age or any other cause, for complete regeneration, there will be no cure; all that can be hoped for is that the disease may be arrested, and this object will best be attained by limiting, by means of a strict diet, the work of digestion. Such a degree of improvement is almost always possible. The morbid process in sprue is distinct from the destructive lesions which it involves: the one reacts to treatment, the other is largely incurable.

The onset and course of sprue are therefore remarkable for insidiousness and chronicity. The disease commences with some form of intestinal irritation,—with biliousness, or dysentery, or the like. Its favourable course depends upon the amount of irritation which it is spared. It is easy to arrest, and difficult to cure. If steps are not taken at least to arrest it, it is progressive and ends fatally; but the disease itself does not kill; death is from inanition or intercurrent infection.

The course of sprue is bound up with the question of treatment, and its bearings upon etiology will be discussed under that head: but the contrast between the original disability and the secondary phenomena which so readily occur: the intimate association of the disease with all kinds of non-specific intestinal irritants; and the insidious commencement and frequent but inconstant latency point, I think clearly, to some other origin than the invasion of a specific organism.

(vi) Treatment. (a) Dietetic. The treatment of sprue, once considered hopeless, is now very fairly satisfactory. Thus out of 100 cases Begg claims 63 cures, with 23 unreported results and only 8 deaths. The figures given by Ashford are almost the same. Rogers, however, considers that a large proportion of such reported cures should properly be classified only as 'improved,' for they remain liable to relapse. But improvement at all events is to be expected in the great majority of cases, and it commences usually with dramatic rapidity. Failure with a sprue case is in fact, to be attributed not to inefficient methods of therapy, but to the difficulties in carrying them out, for they are tedious and irksome. Sprue-patients as a rule are hard to manage; they are irritable and querulous, and often disobedient or unreasonable. Success is directly proportional to the degree of intelligent co-operation of the patient, and



to the facilities for enforcing treatment. The method of such treatment is not indeed the subject of universal consent. Some physicians ascribe their cures to drugs and vaccines; and though most are agreed that the essential treatment is dietetic, there are differences of opinion as to its composition. This is natural enough: individual peculiarities, both in patient and doctor, are seldom more divergent than upon a question of diet: and in sprue especially a diet suitable for one case will fail with another, which will be found quickly to improve if the diet be changed. As to the essential principles of a successful dietary however few will quarrel. In the words of Dr. Carnegie Brown, 'they may be summarized as the adoption of a definite and rigid dietetic regimen. It is imperative to supply the patient with food which, while containing sufficient nourishment for the maintenance of the vital process, shall afford the maximum amount of rest to the digestive apparatus, and the residual products of which shall, as far as possible, be an unfavourable medium for bacterial growth.' The 'milk-cure' is the standard application of these principles, though some physicians claim to be equally or more successful with diets of fruit or meat. The relative merits of these need not now be discussed: from the point of view of etiology the essential point is that by a suitable diet sprue, even though severe, can be arrested and generally cured. This appears to be a very remarkable fact: for milk, or the other diets in vogue, can be considered a specific remedy only in so far as they form a medium 'in which,' as Ashford observes, 'Monilia does not flourish, and which it cannot ferment.' The symptoms of sprue, diarrhoea, flatulent distension, and possibly stomatitis, are due to fermentative action of fungi, and if these are starved out the disease cures itself. From this fact Ashford deduces that sprue is essentially a mycosis; but the conclusion does not seem necessarily to follow. Monilia fungi may disappear from a patient's stool; the results of their activity may be cut short; the mouth-sores may heal up and the diarrhoea cease; weight may be put on and the general health improved; but the disease is not thereby cured. This is the point insisted upon by Rogers, and it appears to be of the greatest importance. For months the stool may remain pale and copious and undigested. During that time and for an indefinite period afterwards any indiscretion in diet may determine a relapse. Some authors, notably Cantlie<sup>(12)</sup>, state even that sprue is never cured by a milk diet, but only arrested and kept in check. This is probably an overstatement; but at least it emphasizes the fact that cure does not depend only upon the termination of the mycotic infection. Castellani



and Low (<sup>1c</sup>) have shown that *Monilia* may be killed by alkaline medication on an ordinary diet, while the disease continues and even progresses. The efficacy of the milk treatment, continued long after the disappearance of all fungi from the stool, clearly depends partly upon other factors. Milk has another property; it throws a minimum strain upon the digestive organs and does not irritate an inflamed and atrophic intestinal wall. By maintaining such a diet the diseased mucous membrane and the exhausted secretions have a chance of recuperation. Were it not so in fact, a milk diet would be most unsuitable in sprue: for the fat that it contains reappears almost quantitatively in the bulky, greasy stool. A protein dietary, such as Cantlie's 'meat-cure' would alone be rational. This cure indeed is often clinically successful; but it has the disadvantage of stimulating digestive activity and peristalsis in organs which above all things require rest. The rationale of the milk diet is therefore incompletely explained by its unfavourable influence upon *Monilia* growth; its second function is scarcely of less value. And while on the one hand the success of this diet testifies to the importance of such infection in producing the symptoms of sprue, it indicates on the other that infection is not the only nor the essential element in the production and progress of the disease.

If the results of diet therapy in sprue are opposed to the theory of a mycotic cause, they speak still more strongly against the probability of its causation by other specific organisms. Specific infective disease may show a long latency and run a chronic, a febrile, relapsing course, as in syphilis; their action may be confined to a single system, as in amœbic dysentery; they may progress by gradual stages and periodical intermissions to a fatal termination as in sleeping sickness: but when, in addition to all these peculiarities, a disease is found to be in all its stages amenable to a simple non-specific treatment, for prompt relief and eventual cure, and up till then to be liable to relapse as soon as this treatment is abandoned, then the conception of a specific invading organism as the source of the disability becomes difficult indeed. A fluctuating and inconstant degree of infection, dependent upon, superadded to, but also itself aggravating, an essential condition of physiological failure of the digestive function, is, on the contrary, perfectly consistent with these facts.

(b) *Specific.* While, as a general principle, the success of a milk diet in sprue is recognized, and in many cases no other form of treatment is necessary, a variety of specific methods have from time to time been

advocated, for which it is claimed that they succeed also in refractory cases and advanced stages, and otherwise hasten recovery. Among such specific aids are yellow santonin (Begg, <sup>5</sup>), streptococcal autogenous vaccine (Nicholls, <sup>28</sup>, and Rogers, <sup>30</sup>), Flexner vaccine (Castellani, <sup>13</sup>), emetine (Schmitter, <sup>31</sup>), strawberries (Thin, <sup>36</sup>), and pancreatine (Brown, <sup>6</sup>). A remarkable case of a strawberry cure is given by Young (<sup>37</sup>). In addition to these, liver, in the form of puree or curry, is an old and successful native remedy in Ceylon, and chalk, in the form of powdered cuttlefish bones, is a famous cure in Shanghai.

Striking successes have been obtained by the use of all these methods ; but general experience does not support the invariable use of any of them. In all probability, therefore, their action is not on the actual cause of the disease, but on accessory factors happening to be prominent in individual cases. For instance, the 'pseudo-sprue' which Castellani cured by Flexner vaccine, seems likely to have been a true sprue in which bacterial infection of the Flexner type played an important secondary part. In a disease where secondary effects are so universal, most cases will in this sense be 'pseudo-sprue.' In other cases, such as those which supervene upon dysentery, this accessory factor is the actual exciting cause, having determined the advent of the sprue-state.

The importance to etiology of these facts of successful specific therapy is as great in failure as in success ; in partial success perhaps it is greatest of all. If emetine or a vaccine or santonin cure some cases but not all, the respective parasites upon which they act must be considered occasional, but not universal, and therefore not essential influences in the disease. They may be predisposing agents ; they may be complications ; but they are not likely to be its actual cause. And, provided the disease is indeed a definite pathological entity, and not an *omnium gatherum* of intestinal infections, the feature common to all cases of sprue alike, that is to say the essential element in the disease, is, it is suggested, in all probability a physiological defect which these variable infective processes have thrived upon, or served to induce or to aggravate.

(vii) Summary. It may here be convenient to summarize the outstanding characteristics of sprue, mentioned in the preceding pages. The most remarkable facts of the disease, then, are as follows :—

- (a) Sprue is a disease of adults ; young children are relatively immune.
- (b) Sprue is a disease of Europeans ; Asiatics are relatively immune.

- (c) Sprue is a disease of the acclimatized; newcomers to the Tropics are relatively immune.
- (d) The geographical distribution of sprue is very wide, spreading over countries not according to geographical contiguity but according to certain climatic conditions. These conditions are those most exhausting to glandular physiological vitality.
- (e) The symptoms of sprue are those of mycotic fermentation; but, when this has been arrested, of faulty fat-digestion.
- (f) Mycoses are common, and do not themselves necessarily lead to sprue symptoms.
- (g) Sprue is clinically indistinguishable from hill-diarrhœa, of which it is not infrequently the sequela.
- (h) Post-mortem, many secondary changes are seen in sprue; anæmia; wasting; an atrophied intestinal wall; an atrophic or cirrhotic liver and pancreas; but no essential nor characteristic lesion.
- (j) The course of sprue is essentially chronic, with a strong tendency to relapse. If untreated, it is slowly progressive, and ends fatally.
- (k) To simple dietetic treatment, however, sprue responds to an extraordinary degree. Such improvement at first is symptomatic only. The eventual cure is long delayed.
- (l) Success has sometimes been obtained with various specific methods of treatment in sprue; each may have value in certain cases, but none are successful in all.

According to the prevailing theory sprue is an infection. Each of the above facts separately is with difficulty compatible with such an origin, whether it be mycotic, protozoal, or bacterial: but as a hypothesis for the correlation of them all, the infective theory appears singularly inadequate; nor is there any positive evidence in its favour. The relative immunity of children, and of newcomers to the Tropics, the affinity with hill diarrhœa, and the prompt response to simple treatment, seem especially incompatible or irreconcilable with a specific infection.

An attempt has been made to point out seriatim that all the phenomena of sprue indicate, not an essentially infective process, but a physiological process; the remainder of the thesis will therefore be devoted to developing this possibility on its positive side.

## V. SPRUE AND FAT-DIGESTION.

The part which infection plays in the disease is undoubtedly very great. Infection is responsible for many of the symptoms: infection may determine the onset: infection no doubt hastens the fatal issue. It is however possible to recognize a stage in sprue when the obvious influence of infection is no longer present; and it is in this stage that the essential nature of the disease lends itself most favourably to study. The stage has already been referred to as that of convalescence. The sprue patient, with a history of months or years of diarrhoea, flatulence, and sore mouth, has been placed on a milk diet and all these symptoms have disappeared. He feels well, looks well, and is putting on weight steadily, if not rapidly. The mouth is clean, and all abdominal discomfort has passed away. He passes only one or two stools daily; often, in fact, he is constipated. The only sign of the disease is the appearance of the stool, which remains pale and fatty. The physician recognizes that he is liable to relapse, and slight relapses may from time to time occur. The importance of this stage in sprue has not, I think, been duly appreciated. It may slowly pass off, the stools returning to normal, and normal digestive power being slowly regained. If the disease has been advanced, or the patient elderly or feeble, it may be permanent. In such cases indiscretion in diet will probably follow sooner or later, and a disastrous relapse will occur: if the diet be strictly followed, good health may be indefinitely prolonged. The patient will then usually discover for himself the limits of diet which he cannot exceed with impunity.

This state, whether it be temporary or permanent, would seem to be the typical sprue-state, shorn of all accidents and complications. Its significance moreover is clear: it is a condition of deficiency in digestive power, more especially in the power to assimilate fat. This failure of a physiological function is therefore suggested as the fundamental defect responsible for sprue; and it remains to analyse in greater detail the fat-digesting function in the light of the different phenomena of the disease which have been enumerated, in order if possible to define the link or links in that process, whose absence or insufficiency might be held to constitute the onset of sprue.

The digestion of fat in the stomach is a negligible quantity: the fat-digesting mechanism in the intestine alone requires consideration. Further it is possible at once to dismiss the possibility of gross pancreatic disease as a common or principal cause of the condition; for though in the advanced stages of cachexia that are seen in the post-mortem room

the pancreas may often have been found atrophied or cirrlosed, many instances are also on record where it has been found perfectly normal. Thus limited, the possible causes of physiological failure in sprue are (1) defective supply of pancreatic lipase from functional inhibition or exhaustion, (2) defective absorption by the intestinal mucous membrane, (3) defective supply of bile, whose salts, as is well known, play an essential part in fat-digestion, (4) defective supply of secretin, the normal chemical stimulus both to pancreatic and to hepatic secretion.

(1) Failure of absorption. The process of fat-assimilation consists firstly in its emulsification, secondly in its saponification. Absorption takes place in the saponified state. The proportion of saponified fats to neutral fats in the stool might therefore be a valuable index as to whether a fatty stool was due to defective digestion or defective absorption. The complicating influence of fat-splitting organisms, however, limits the practical application of this test, and the copious analyses of the sprue-stool recorded by Cammidge <sup>(11)</sup> are therefore difficult to interpret.

Impairment of absorption in active and advanced stages is likely enough. The widespread erosion of mucosa must materially limit the absorbing surface; the more so because much of the mucous membrane not actually ulcerated is coated with a thick layer of viscid adherent mucus. These effects would be less marked in early cases, when typical sprue-stools are passed, and during convalescent treatment, when fat is still abundant in the fæces.

On other grounds, however, than fat-analysis of the stools, it is clear that deficient fat-absorption is not the only factor in sprue. This does not explain the colourlessness of the motions, nor why fermentative processes should be so especially virulent. It does not explain the diminution in size of the liver, nor the remarkable success of a milk-diet. More important still, it affords no explanation of the sprue-like symptoms of hill-diarrhœa, nor for the characteristic tendency of sprue cases to sudden relapse; for the power of absorption is not a function likely to become suddenly arrested, nor to exhibit violent fluctuations.

(2) Failure of bile-salts. Besides the presence of fats in the stool, other cardinal signs of sprue, namely, the colourlessness of the stool, and the diminution of liver-dulness, are suggestive of a functional derangement of the liver, possibly a physiological exhaustion. This is supported by the evidence of the preliminary stage of bilious diarrhœa, so often a feature of sprue-history. The facts indeed indicate with certainty that

the liver is not secreting normally in sprue : even that failure of bile is characteristic of sprue : yet there are several reasons for not attributing the disease entirely to this cause. A. Bile and bile-salts may be absent from the bowel in diseases other than sprue ; for instance, obstructive jaundice. The stools in this condition do indeed resemble those of convalescent sprue : they are bulky, greasy, pale, and alkaline. But in jaundice there is none of the extreme sensitiveness to changes of diet, which is so characteristic of sprue : there is no intolerance for carbohydrate : and there is no tendency to aphthous infection or fermentative decomposition. B. Bile, if deficient, can readily be replaced by bile-feeding. Improvement by this treatment is said to occur in some cases of sprue, but even in convalescent cases, when infective elements have been eliminated, the administration of bile does not usually alter the character of the stool, nor avert the tendency to relapse.

C. Bile-pigments are not absent from the sprue-stool, though doubtless deficient in quantity. Traces of bile-salt have also been found. (This would indicate a faulty absorption, for the bile-salts are not normally excreted.) Post-mortem, bile is found in abundance in the gall-bladder and small intestine. The colourlessness of the stool is due, not to absence of bile, but to the chemical conversion of the bile pigment into a colourless form, and to the high proportion of fat in the stool.

(3) Failure of the pancreatic juice. Positive evidence of the presence of this factor in sprue is afforded by the following facts :

A. Deficient digestion of carbohydrates. The efficacy of the milk diet consists not a little in its poverty in carbohydrates. For the same reason sour milk, in spite of its acidity, is often more effective than fresh milk ; in one of my cases, an elderly man, this was very marked.

B. Bleaching of bile-pigment. This process, already mentioned, is said to be due to the absence of pancreatic ferment (Harley and Goodbody).

C. Absence of trypsin from sprue-fæces (Bahr). Faulty protein digestion is also indicated by an excess of faecal nitrogen (Harley and Goodbody). This effect is the more remarkable as occurring on a diet so easily assimilable as milk-protein. Brown (7) also records absence of trypsin, and indeed of all pancreatic ferments, in one of his cases.

D. Benefit from pancreatine. In the same case described by Brown, the administration of pancreatine promptly checked the diarrhoea, which recurred directly the drug was omitted. I have had a similar case, in which there was no reason to suspect pancreatic cirrhosis : though



others have had a different experience. Bertrand and Fontan strongly advocate this form of therapy ; in some cases it is certainly beneficial.

E. Chemical tests for pancreatic insufficiency. The Cammidge reaction is said to be positive in a certain number of cases ; though evidence from this uncertain test must be received with caution. I have found no increase in the urinary diastase.

(4) Failure of the chemical hormone secretin. This substance, like enterokinase, is formed in the mucous membrane of the duodenum and jejunum ; unlike it, secretin is not discharged into the bowel, but absorbed into the blood-stream, by means of which it reaches, and serves effectually to stimulate, both the pancreas and the liver. From failure of secretin the supply of lipase, diastase, trypsinogen, and bile would also partly fail, without organic defect or abnormality of the liver and pancreas.

Little is known of the physiological and pathological conditions of secretin-formation, but the possibility of its failure in sprue would account well for the immunity of children, in whom secretin-production is very abundant, and for the effectiveness of milk diet and a change of climate. The similarity of sprue and hill-diarrhœa, also, would be easily explained. Indeed the whole clinical picture of sprue would be readily intelligible, if this hypothetical cause could be verified.

Unfortunately it is difficult to put this attractive possibility to the test. The intravenous administration of secretin is uncertain and dangerous, and when given by the mouth it is unabsorbed (Starling <sup>32</sup>). I have tried its use in sprue-cases without any benefit. There is no positive evidence moreover, that the supply of secretin in sprue is curtailed.

If an analysis of the physiological breakdown in sprue be attempted therefore, the following conclusions are obtained :—

(a) Defective absorption, as opposed to defective digestion, is an important complication of advanced cases : but it is not an essential feature of the disease, for it is absent in early stages, and in hill-diarrhœa.

(b) Defective secretion of bile and pancreatic juice is present in all cases : it may depend upon primary exhaustion of the liver and pancreas, or possibly it may be due to

(c) Defective output of secretin, the normal chemical stimulus of their action.

#### *Sprue a climatic disease.*

Though the phenomena of sprue-convalescence point to the essential presence of a physiological deficiency, this deficiency might possibly be



thought to be the persistent effect of the disease rather than its cause. Further evidence of the defect must therefore be sought in an earlier stage, before infective elements have developed, rather than after they have disappeared. Such evidence, I think, is afforded by the clinical condition of hill-diarrhœa, already briefly described. This disease, I have ventured to suggest, represents an acute form of sprue, produced by acute climatic change. The physiological disturbance in which it consists, usually subsides after removal of the cause, or on acclimatization: occasionally the symptoms persist, or, in other words, ordinary chronic sprue supervenes. In all cases an instance is afforded of the digestive incompetence characteristic of sprue, arising in the absence of an infective cause. In connexion with the suggestion already made, that the exhausted function may be that of secretin-production, rather than the primary activity of liver or pancreas, it is perhaps worthy of note that the only pathological lesion found in hill-diarrhœa has been an intense congestion of the intestinal mucosa.

A physiological breakdown is perhaps the oldest of all theories of sprue. Its revival is suggested as a deduction from the clinical condition of sprue-convalescence. But it harmonizes also with most of the other phenomena of sprue which have been mentioned. The age and race incidence, the latency, the course and the relapses, the inconstant pathological findings, the symptoms, and the effects of dietetic and specific treatment, are simply explained. Two characteristics alone are difficult to understand: the peculiarities of geographical distribution, and the prevalence of mouth-symptoms.

The details of geographical incidence of sprue are as yet insufficiently well-known. Certain anomalies seem definitely to exist. But since, generally speaking, sprue is so obviously associated with a certain type of climate, it must be considered highly premature to conclude, as some have done, from minor irregularities in this distribution that climate is not responsible for the disease. The exact nature of the influence of climate upon physiological processes is as yet an almost entirely unknown and uninvestigated field.

The nature of the mouth-symptoms in sprue is of greater immediate importance; and at risk of repetition the outstanding facts about these symptoms may again be enumerated.

1. They are associated with sprue, constantly in Ceylon, the Malay States, and China; frequently, but not always, in India and elsewhere.

2. They may occur without other stigmata of sprue, especially in Ceylon and among lower-class natives.

3. Subjective intolerance for spicy foods is a prodromal symptom, indicating early denudation and exposure of the taste buds. This is likely to be due to the especial sensitiveness of these organs to inflammatory change.

4. Friction exerts an important influence, causing early involvement of the edges of the tongue (Crombie's ulcer).

5. Eventually the whole of the buccal epithelium may be extensively denuded.

6. There is extreme liability to fluctuation and relapse, not necessarily proportional to the general progress of the disease. Its severity sometime increases with, and sometimes apparently alternates with, an increase in the diarrhoea.

7. There is extreme variability in the degree of the mouth-symptoms in cases of sprue otherwise similar.

8. The saliva is acid.

9. The symptoms respond quickly, in favourable cases, to mild alkaline antiseptic treatment.

10. The bacteriological findings from scrapings are highly inconstant.

These characteristics indicate, I think, clearly that the condition of sore-mouth is one, not of specific infection, but of lowered resistance. In the absence of all knowledge of the normal infection-resisting mechanism of the mouth-parts, the cause of this lowered resistance must remain obscure. The physiological cause must be specific, for these symptoms are not found in other wasting diseases: yet the lesion itself is not specific, for various organisms may produce it.

It is noteworthy that in the rare and obscure disease of children known as coeliac disease, Still (<sup>31</sup>) has described the occurrence also of a sprue-like stomatitis. Coeliac disease, which is very similar to sprue in other respects, appears to be pathologically a sprue-condition, due to some congenital physiological defect.

The complete description of sprue in the light of the above facts and suggestions would therefore include two conditions. In the first place there is a physiological failure of the fat digesting mechanism. In the second, there is a physiological failure of the normal antiseptic vitality of the buccal mucous membrane. The two processes are usually but not always associated, and either may occur without the other. The

presence of the last-named factor undoubtedly complicates the issue but has attracted perhaps an undue amount of attention. For though it may not as yet be explicable, it affords no evidence for a specific infective causation; but, being demonstrably non-specific, rather the reverse. It may one day be possible to correlate two phenomena of physiological insufficiency; but it is not easy to imagine the association of one phenomenon of diminished resisting power with another of specific infection. If infective elements are secondary in the one, they are probably secondary also in the other. The precise nature of the disability in sprue is not understood, but it is essentially physiological and not infective: the disease follows from deficiency from within, and not from the virulence of external invasion.

#### VI. CONCLUSIONS.

1. Sprue is a definite clinical entity, and not a mere congeries of symptoms. Its predisposing and complicating factors lead to, or depend upon, a single pathological condition.

2. There is no positive evidence to suggest that this is a specific bacterial or protozoal action. Protozoa or bacteria may be present, and their destruction may lead to, or even be necessary for, the cure of sprue-cases; but the sprue-state may exist in their absence.

3. The symptoms of sprue are mainly due to two factors, namely, (a) mycotic fermentation, and (b) digestive incompetence. The two most probable theories of the nature of the disease are accordingly (a) that it is a mycosis, and (b) that it is a physiological exhaustion-state, due to climate. Whichever of these be the primary defect, they operate in a vicious circle. Defective digestion favours the multiplication of organisms, while an infective catarrh accentuates digestive weakness.

4. The peculiarities of the incidence and geographical distribution of sprue point to a physiological rather than to an infective cause.

5. The immediate relief from all infective symptoms in response to non-specific treatment is contrasted with the very slow and uncertain recovery of the digestive function. This also is taken to indicate that the digestive failure is the essential disability, and the infection secondary.

6. The prominence of infective processes in the mouth in most sprue cases, and their tendency to relapse, cannot be explained by any physiological failure of digestion. It is, however, even less easily explicable by any infective hypothesis, for it is in origin physiological, depending essentially upon lowered resistance: the actual infecting organism is accidental and variable.

7. The physiological disability of sprue, manifesting itself thus firstly as a failure of intestinal digestion, and secondly as a state of lowered antiseptic vitality of the buccal epithelium, is brought about by the effects of climate, though the nature of climatic action, and the exact climatic elements responsible, are unknown. In the cœliac disease of children a similar disability is present, but is congenital.

8. Hill-diarrhœa is essentially a form of sprue, acute in onset and uncomplicated in course. This disease affords an example of sprue-symptoms arising in the absence of infective causes.

9. The physiological fault in which all these three diseases consist may lie in the actual digestive glands, the liver and pancreas. Possibly however it should be located in the supply of the chemical hormone secretin, which normally affords to these glands their effective stimulus.

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## REPORT ON HOOK WORM INFECTION IN THE UNITED PROVINCES.

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THE following is an abstract from a report which was prepared by me in September, 1919.

Owing to special circumstances it was drawn up in a great hurry, but the Scientific Advisory Board, Indian Research Fund Association, has suggested that part of it might be of value in stimulating such mapping out in other provinces.

The readers of the Journal will at once understand that the report was not intended for perusal of experts.

Colonel Close, Inspector-General of Civil Hospitals, United Provinces, and the Government of the United Provinces have readily agreed to the publication of this extract.

This report is based on investigations carried out during the past 18 months by Civil Assistant Surgeons L. N. Rai and N. D. Banerji under the control of a committee consisting of the Hon'ble Colonel C. Mactaggart, C.S.I., C.I.E., I.M.S., Inspector-General of Civil Hospitals, Lieut.-Col. Henderson, I.M.S., Inspector-General of Prisons (his place was taken afterwards by Lieut.-Col. J. M. Woolley, I.M.S.), and myself.

The enquiry was sanctioned by the Local Government, which directed that it should be carried out on the basis suggested by the Hon'ble Colonel Mactaggart in a letter dated the 28th May, 1917.

The scope of the enquiry had to be confined to the most strictly essential points owing to the great shortage of medical men in the province caused by the war.

The general scheme of the enquiry and the administrative details connected with it are the work of Colonel Mactaggart. Doctors Rai and Banerji were first sent for a three months' course of instruction in the method of investigating the disease. They had the great advantage of receiving this instruction from Lieut.-Col. Clayton Lane, I.M.S., then Civil Surgeon of Hooghly, who is recognised as the greatest authority on hook worm infection in India.

On the completion of this course they were instructed to enquire into the occurrence of the disease in certain prisons in various parts of the province.

The essential point in the enquiry was to find out the degree of prevalence of the infection in the province, but incidentally it was found possible to collect information regarding certain other points which will be of considerable value in helping us to form a true estimate of the importance of the disease.

After completing the investigation into the prevalence of disease in the jails, enquiry was carried out in the large hospitals of Agra and Lucknow with special reference to the relative frequency of infection among persons living in large cities as compared with persons from rural areas.

An important part of the programme of Doctors Rai and Banerji consisted in giving a course of instruction in the diagnosis and treatment of hook worm disease at selected centres in the province.

This method of investigation was that of Lieut.-Col. Clayton Lane; it consists of a microscopical examination of stools which are treated in a special way for the purpose of separating the eggs of the hook worm as far as possible from the other materials in the stools.

The method is so delicate that it reveals the existence of infection in a much larger percentage of people than was the case with the older methods.

Table I shows the degree of prevalence of the infection in the province and it will probably cause surprise to most people to learn how widespread and how common the infection is over the greater part of the province. The figures suggest that nearly three-fourths of the adult population are infected with hook worms.



The figures for the tahsils of each district were kept, but as they seldom show any striking divergence from the figures for the district as a whole, they are not given separately. Besides, if small numbers of examinations are dealt with, the element of chance enters so largely into the results that they are of much less value than when large numbers are available.

It will be seen that certain districts are comparatively lightly infected. These are the districts situated in the south-western part of the province :—

Jhansi	..	..	..	..	7 per cent	infection.
Banda	..	..	..	..	23	„ „
Cawnpore	..	..	..	..	30	„ „
Agra	..	..	..	..	34	„ „
Etawah	..	..	..	..	37	„ „

It is likely that these districts are less intensely infected because the conditions of climate and soil are unfavourable to the spread of the infection, as there is no observable difference in the manners and customs of the inhabitants which is likely to affect the probability of infection.

As the eggs occur in faeces and can only be hatched out in the presence of moisture it is likely that dryness of the soil is an important factor.

In Jhansi 50 per cent of the soil is said to be black cotton soil which becomes much fissured by deep cracks in the dry weather, so that, probably, the surface will be exceptionally dry for a great part of the year. Most of the rest of the soil of Jhansi is sandy and very pervious to moisture. Jhansi district as a whole is 'everywhere excessively drained.'

In Banda the prevailing soil is a light sandy soil, next in extent comes a soil rich in kankar; both of these soils are likely to be dry. Besides, Banda is said to 'suffer much from overdrainage,' and so there is likely to be very little opportunity for the soil to become richly infected with the embryos.

In Cawnpore district 'The drainage is generally good.'

In Agra 'The bulk of the district does not suffer in any way from defective drainage.'

In Etawah 'The general excellence of the natural drainage is exemplified by the general rarity of lakes and marshes.'

The above notes regarding the soil are taken from the District Gazetteers.

The map shows in a graphic manner the intensity of infection in the different districts where the examination was made.

The influence of rainfall is not very striking, though from Table III it will be seen that on the whole a low rainfall is usually associated with a low rate of infection and *vice versa*.

It is probable that the amount of water that remains in the surface of the soil matters much more than the amount that falls.

A heavy rainfall might even be useful in washing away the infection, provided that there is a free outlet for the surface water.

A close survey of a number of places which show a very high and a very low rate of infection would probably yield results of considerable interest and importance.

Factors such as the duration of drought, soil temperature in the hot weather, types of vegetation at various seasons would have to be taken into account.

Table IV shows the influence of prison life on the infection. It was considered likely that the figures in Table I would be open to criticism on the ground that they dealt only with persons in prison and that they might merely indicate that prisoners become infected in jail.

Though such a suggestion would have been contrary to all previous experience of the disease, it was thought advisable to place the facts on record.

It will be seen that the infection tends rather to die out in jail, and probably one of the factors that explains the improvement in health that usually follows from jail life is the comparative freedom from re-infection that is enjoyed by the prisoners.

From the point of view of the present enquiry the important matter is that figures obtained from the jail population are likely to lead to an underestimate rather than to an overestimate of the general prevalence of the disease.

#### INFLUENCE OF THE INFECTION ON THE GENERAL HEALTH.

To those who have followed the records of the many investigations into this point there is no need of further evidence as to the harm that results from hook worm infection.

In spite of this the figures in Tables V and VI are interesting as showing that there is definite evidence of a deterioration of the general health in this province, even by slight and moderate degrees of infection. The chart (Table VII) shows the same thing in a graphic manner.

The records of the prisoner's health were taken directly from the prisoner's history sheet, in the case of Dr. Rai's enquiry, so there is no possibility of the classification being biased by the investigators.

The state of health had in each case been entered by the Superintendent on the admission of the prisoner to jail.

In the case of Dr. Banerji's figures the note as to the prisoner's state of health was made independently of the results of the examination of the stools, but in some cases Dr. Banerji was aware of the results before recording the prisoner's state of health. The close correspondence between his results and those of Dr. Rai shows that he managed to avoid bias, even of an unconscious nature.

In the column dealing with 'Apparent anaemia' as judged by inspection of the conjunctiva and lips there is one discordant figure in Dr. Banerji's record. This does not affect the general results, and it is of little importance as an inspection of this kind cannot be expected to convey an exact estimate of the degree of anæmia. Even considerable degrees of anæmia are liable to escape detection if mere inspection is relied on.

An interesting and important fact is shown by Table VIII, *viz.*, that those who live in towns and cities are much less liable to infection than those who live in the country.

The probable explanation of this is that the use of latrines and the regular removal of night-soil greatly reduces the soil contamination, in spite of the fact that the population is much more densely collected in towns. On the other hand, it must be borne in mind that the occupations of those who live in towns are such that they are not brought into so close contact with the soil as those who live in the country.

The figures for towns and cities would probably be still better than they are, but for the fact that many people in the outskirts of the towns live in conditions very similar to those prevailing in the country.

Besides this, many of the persons who describe themselves as dwellers in towns, spend a great part of their time in the country. It is likely, too, that the latrines in most towns and the night-soil removal are far from perfect.

It is not possible at the present time to assert that the introduction of latrines and night-soil disposal would cause a great diminution in the infection rate for villages and rural areas, though there is every reason to hope that they would do so.

Table IX shows the influence of occupation.

The figures tend to show that people who come in contact with the soil are more liable to infection than those whose work is in the shop or workroom. These figures may however be misleading as there are more town dwellers among the artisans and their relative immunity may be attributed to town life.

In fact it is necessary to maintain a strictly critical attitude with regard to all the figures and to keep a careful look out for factors which may not be apparent at first sight.

#### *Influence of sex.*

Of 185 females who were examined 112 were found to be infected, viz., a rate of 60·54 per cent against a rate for males of 74·34 per cent. This indicates a slight relative immunity of females which is probably due to their being less frequently in the fields than the males.

#### *Influence of religion.*

In a large series of cases it was found that 88·04 per cent of the Hindus were infected against 60·38 per cent Muhammadans.

Dr. Rai suggests that the Muhammadans escape to some extent because of wearing shoes.

### SIGNIFICANCE OF HOOK WORM INFECTION IN THE UNITED PROVINCES.

The Tables show clearly that hook worm infection is exceedingly prevalent in the United Provinces.

In most of the districts it is reasonable to assume that nearly every one who has reached adult life harbours hook worms or has some time harboured them. It is important to try to form a definite opinion as to the effect of the infection on the individual and on the population of the province as a whole.

In many parts of the world enquiries have been carried out by experts with special reference to this point, and the generally accepted view is that even in slight infections an appreciable diminution in health and energy is caused by the hook worm.

Severe infections, on the other hand, cause serious anæmia and in many cases death. *Prima facie* we may assume that such a high rate of infection as is shown to occur in the United Provinces indicates that

the following consequences are likely to follow from hook worm infection in this province :—

I. A general diminution of the standard of health and energy among the infected, and consequently in the aggregate a great economic loss to the province.

II. A large number of cases of serious illness due to the action of the hook worm.

Many of these cases of illness are likely to prove fatal either directly from anæmia or indirectly from some other diseases from which the patients would have recovered but for their vitality being lowered by the hook worm infection.

III. The birth rate is likely to suffer, as it is known that infection of the mother greatly predisposes to miscarriage and premature labour.

IV. Apart from the effect of infection on the adult, there is likely to be a serious impairment of physique due to the malnutrition caused in the mother during the period of gestation and suckling and due to the harmful effects of the infection on the individual during childhood. Severely infected children show a remarkable degree of defective development; they are puny and stunted and are of defective intelligence.

The above conclusions regarding hook worm disease are now generally accepted and further investigation into the matter would seem to be almost superfluous.

Still, it is worth while to see whether there is any evidence that such conclusions are justified in so far as this province is concerned.

The figures shown in Tables V and VI have been worked out independently by Dr. Rai and Dr. Banerji. In the case of Dr. Rai's figures the condition of the prisoner's health was that stated on the history tickets, so that there can be no possibility of personal bias.

Dr. Banerji examined the prisoners himself, but as his figures show essentially the same results as Dr. Rai's, he appears to have avoided the unconscious bias that is liable to creep into such an investigation.

These figures are likely to give an underestimate of the injury done by the hook worm rather than an overestimate, for the slight and moderate degrees of anæmia caused by the hook worm are not associated with wasting or other apparent loss of health and a much better idea of the disability caused by the hook worm is obtained by the comparison of the work done by voluntary labourers before and after being freed from hook worms. In the Porto Rico experiments the increase in the output

of work after treatment varied from 30 per cent to 100 per cent in the case of workers on the coffee plantations.

The figures of the birth-rates and death-rates in the districts of the province are so greatly influenced by other factors that it is hardly possible to draw satisfactory conclusions from them.

In two of the districts which show a low rate of infection there is a great preponderance of births over deaths during the years 1891 to 1900, but in two others, Jhansi and Banda, the deaths are actually shown as being in excess of the births. Such factors as famine, cholera, plague and imperfect registration render the official figures useless as an index of the influence of hook worm infection on the birth and death-rates.

*Evidence from other sources.*

From the records of the King George's Hospital, Lucknow, it appears that during the past two and a half years 46 cases of hook worm anæmia were treated as in-patients in the hospital; 22 of these came from the Lucknow city and district. The diminution in the colouring matter of the blood in these cases varied from 50 per cent to 85 per cent and on the average each patient was found to have only 34 per cent of the normal amount of colouring matter in his blood.

When we consider that in the year 1918 there were only 18 proved cases of malaria treated as in-patients in the same hospital it is clear that hook worm anæmia is by no means a negligible disease in the province.

Account must be taken of the fact that Indians suffering from medical diseases are as a rule very reluctant to enter hospitals for treatment.

The hospital records only show severe cases; for every person suffering from severe anæmia there must be many persons suffering from lesser degrees of anæmia; these do not think of coming to hospital for treatment.

The hospital records thus fully corroborate the findings of Dr. Rai and Dr. Banerji and it may be regarded as proved that there are many cases of severe anæmia in the province; a considerable proportion of these die either of the anæmia or of some other disease which in itself would not prove fatal.

There must also be an enormous number of people who suffer from lesser degrees of anæmia and from corresponding degrees of mental and physical inefficiency.

If the most conservative estimate of the average loss of efficiency is made and it is assumed to be as low as 20 per cent. it still represents a very serious economic loss to the province, apart altogether from the deaths and sickness caused by the disease.

In the modern industrial competition such a loss of efficiency is well worth the attention of those whose duty it is to help India to improve her economic condition.

Throughout the enquiry an attempt has been made to collect facts, and it is claimed that the figures shown in the Table represent facts and give a fair idea of the state of affairs existing in the province.

In the deductions that have been made from those facts the personal element enters and there is room for difference of opinion, but every effort has been made to avoid overstating the case, and conclusions have been stated only when the evidence appeared to point irresistibly towards them.

#### WHAT ARE THE MEANS OF PREVENTING HOOK WORM DISEASE

It is believed that the universal use of suitable latrines or suitable septic tanks will prevent the soil from being contaminated by the infected stools and so the disease will be stamped out.

It is beyond the scope of this report to discuss the best forms of latrines for villages in the United Provinces; indeed it is doubtful whether a definite reply could be given off-hand if any one were to ask for a design which would prove in every way suitable. In the meantime it is likely that even a properly constructed cess pit protected by a grass roof and provided with wooden platform and lid would lead to a great degree of improvement.

In a recent medical journal a writer on hook worm infection in China was much exercised in mind because he found 13 per cent of the population infected. He considered that the use of feces as a manure was chiefly responsible for this high rate of infection. The Chinese system is to use cess pits and to employ the contents as manure, but considering that the infection rate was so much lower than in most parts of India, it is quite likely that the use of the cess pit would be a great improvement on the existing Indian method, and if combined with some safe means of disposing of the contents it might even form a solution of the problem.



Apart from the difficult and unsavoury problem of disposal of fæces there is another possible way of dealing with the infection. This is more likely to appeal to the individual as it combines cure and prevention.

It is found that certain drugs like Thymol and Oil of Chenopodium when taken by the mouth can cause the worms to let go their hold on the intestine, and if these drugs are combined with suitable purgatives they cause the expulsion of the worms.

In most cases two courses of treatment will clear the intestine of all the hook worms. Unfortunately the treatment does not prevent re-infection, so that, unless soil infection is guarded against, fresh parasites will soon take the place of those that have been expelled.

#### WHAT ACTION IS CALLED FOR TO DEAL WITH HOOK WORM INFECTION IN THE UNITED PROVINCES ?

I. One important step has already been taken.

On the suggestion of Colonel Mactaggart the committee decided on a course of instruction in hook worm disease for the medical officers of the dispensaries of the province.

The classes were held at convenient centres by Dr. Rai and Dr. Banerji, who gave a short course of instruction in the diagnosis, treatment and prevention of hook worm disease to such Assistant Surgeons and Sub-Assistant Surgeons as could be spared.

Altogether 45 Assistant Surgeons and 205 Sub-Assistant Surgeons and three private practitioners attended the lectures. It is to be regretted that some of the Assistant Surgeons did not make a special effort to avail themselves of the opportunities afforded by the classes, but there can be no difference of opinion as to the value of the course of instruction in calling the attention of doctors to the existence of the disease and to the modern methods of diagnosis and treatment.

In my opinion classes of this kind might with great advantage be held at intervals, to bring medical men into touch with recent developments in connection with such diseases as malaria, dysentery, kala-azar, tuberculosis, venereal diseases, etc.

Selected men might be deputed for a course of post-graduate study of a particular disease, they could then make an inquiry into the prevalence of the diseases in the province and into the important local factors connected with it. Having done so they could hold short courses of instruction in selected centres just as was done in connection with hook worm disease.

Such itinerant post-graduate instructors would serve a very useful function in keeping up the knowledge of medical officers of dispensaries all over the province.

It is easy for us to lay stress on the necessity for reading medical journals and recent medical books, but the doctor who is cut off from association with other medical men and who is overwhelmed with work cannot be blamed if he loses his early enthusiasm. Almost without exception he becomes a victim of routine, and courses of instruction of the kind suggested will constitute a very valuable corrective to the tendency to degeneration.

A word must be said for those who may be deputed for special duty of this kind.

Their work is arduous and involves discomfort and risk to health. It is essential that there should be liberal treatment in the matter of pay and allowances, otherwise the best men cannot be expected to take up the work with enthusiasm.

Work of this kind should be entrusted to picked men, who should be made to feel that their work is valuable and that they are specially favoured by being selected to do it.

II. The next step that should be taken is to supply the dispensaries with the means of diagnosis of the disease.

Several naked-eye methods of diagnosis have been tried by many investigators. Some of the most likely were tried by Dr. Banerji, but the results were not satisfactory.

It is generally agreed that a microscope is essential for any one who wishes to attain to accuracy in the diagnosis of the disease.

The instrument need not be an expensive one, and a suitable one could be obtained at a cost of about Rs. 75 to Rs. 100. But as a medical man without a microscope is like an astronomer without a telescope, there is not likely to be any opposition to the supply of microscopes even to the smallest dispensaries.

Without a microscope the doctors of dispensaries are likely to forget what they have learnt and to lose the benefit they have gained from the recent classes.

III. A further step is to see that a good supply of the necessary drugs is available at the dispensaries.

I do not know of the conditions prevailing in the United Provinces, but in certain other provinces the small dispensaries and even some of the

larger ones used to have a grant for drugs which was lamentably inadequate.

It would be injudicious to sell treatments at post offices.

Indiscriminate administration of Thymol and Oil of Chenopodium is dangerous.

Unpleasant symptoms and even death follow at times from the administration of these drugs.

The drugs are ordinarily quite safe, but in the case of very enfeebled and anæmic persons they may cause poisoning.

Besides, there are certain precautions that have to be observed in connection with the use of the drugs.

It would be safer to insist on the persons treated remaining under observation for the rest of the day, except in the case of persons who are in good general health and are not enfeebled by the disease.

IV. The next step is to instruct the people in the nature of the disease and in the means of dealing with it, and of course such instruction should not be confined to the hook worm disease; there are many other diseases even more important, *e.g.*, tuberculosis, malaria, plague, venereal diseases, cholera, relapsing fever, and it is a matter of some urgency that the mass of the people should be given the benefit of the knowledge that has recently been accumulated by medical scientists.

The most obvious ways of instructing people consist of (1) lantern lectures like the one written by Lieut.-Col. Clayton Lane, (2) illustrated leaflets, (3) illustrated posters in railway stations, post offices, schools, etc., (4) articles in newspapers and journals.

The *United Provinces Journal* is an excellent medium for the spread of knowledge of preventible diseases, but its circulation should be greatly increased.

V. The treatment and prevention of the disease should be carried out in prisons, police lines, schools and other institutions, where it is possible to maintain control and supervision.

VI. Then experiments should be carried out in selected villages on the same lines as were followed by the Rockefeller Foundation in Ceylon tea gardens and elsewhere or on the lines followed by Lieut.-Col. Clayton Lane in the tea gardens in Assam. The difficulty of such experimental work due to indifference and misrepresentation should be clearly recognised and guarded against.

The general lines to be followed in such experiments are—

(a) Instruction of the people concerned.

- (b) Examination of the whole population included in the experimental area.
- (c) Treatment of those who are found to be infected.
- (d) Preventive measures, especially the introduction of suitable latrines.
- (e) Subsequent enquiry as to the results, especially with reference to the degree of reinfection.

VII. A safe and useful step which can be taken at once is the extension of sanitation to the towns and larger villages \* which have not yet adopted latrines and a proper system of night-soil disposal.

Even for this step a necessary preliminary is the provision of a pattern of latrine which has been proved to be satisfactory.

The foregoing measures can easily be carried out and they should be adopted at once, but they do not constitute a complete solution of the problem, in fact they merely touch the fringe of the question.

Nothing short of eradication of the evil should be aimed at.

It is when we come to devise a working scheme for dealing radically with hook worm infection in the province that we are confronted with difficulties which are insuperable at the present time.

Until the masses come to realize the harm that is done by hook worm infection and are ready to co-operate in the measures that are necessary for dealing with it, no great improvement is possible.

In dealing with hook worm infection the sanitarian is met with the same difficulty as confronts him in dealing with the problem of tuberculosis, malaria, plague, cholera and other diseases; but whereas the evil effects of many other diseases are obvious to every one, they are by no means striking in the case of hook worm disease.

If such limited success has been attained in combating diseases whose effects are sudden and sharp and obvious, there will naturally be much greater difficulty in securing co-operation against so insidious an enemy as the hook worm.

Much has been found out about the means of preventing most of the great fatal diseases: very little of the accumulated knowledge has been translated into practical action.

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\* In the report the wording was 'to villages and towns.' This wording suggested to some, that I advocated an extension of the use of latrines to small villages and it was pointed out that this is impracticable.

My idea is that the use of latrines could be extended to smaller centres of population than possess them at the present time.

It is easy to arrange for propaganda in the form of leaflets, lantern lectures, etc., but it must be confessed that they have hitherto failed to produce the results that have been hoped for ; they have made but a faint impression even on the scanty audience which has been reached by them.

It is important that this comparative failure should be frankly recognised and that the reasons for it should be brought to light.

If we wish to convey a particular piece of knowledge that can only be communicated in one language, the first essential is that our hearers should learn that language ; and it is because the people of India do not understand the language of hygiene that we fail to communicate the knowledge of the prevention of disease to them. If we employ our imagination and try to appreciate the mental outlook of the average villager we may cease to turn away from him in despair and disgust because he does not promptly realize the value of our advice.

The villager has been accustomed for generations to regard disease as a mysterious evil which no human power can prevent. He accepts it with resignation as a piece of ill-luck or as a punishment for his sins. It is not to be expected that he will change his entire outlook on nature in consequence of a harangue which is unintelligible to him.

Another thing that affects the villager's attitude towards the sanitary reformer is that he has learned from experience to distrust the stranger who comes to him with advice. So it comes about that he does not comprehend our lectures on sanitation and he suspects our motives when we ask him to carry out new-fangled suggestions. If he is sufficiently interested as to make further enquiries, he will possibly go to the school master and ask him what he thinks of the strange talk that they have just heard. The school master usually understands nearly as little as the villager, and like most pedagogues he will not care to admit that any subject of which he is ignorant can be of great practical importance.

If we were to carry the defence of the villager's attitude a little further we might suggest that he is justified in arguing that the newfashioned ideas about sanitation cannot be of much practical value, otherwise the school master would know about them and would teach them to the children under his care.

It appears then that it is unreasonable to expect the mass of the people to appreciate the importance of sanitation unless we can entirely change their mental outlook towards disease.

This can only be done by the teaching of sanitation in their childhood.

If even a few people of a village had been instructed in the elements of hygiene, the attitude of the villagers as a whole to the sanitarian would be entirely different. He would be listened to with some degree of understanding instead of with the hostile suspicion engendered by ignorance.

There does not appear to be any prospect of success in spreading a knowledge of hygiene among the people unless it becomes a subject of instruction in schools and colleges.

If this is admitted there only remain two questions to be answered. One is 'Is it worth while'? The answer to this is that it has been clearly demonstrated that attention to the elementary rules of hygiene can diminish the number of attacks of serious diseases by three-fourths, it can prolong the average life of the people by 20 years, and it can produce an enormous improvement in the economic condition of the people.

This is not a rhetorical claim, it is a statement of amply proved facts. Can such a statement be made regarding any of the subjects that are taught in schools at present?

If we grant that the teaching of hygiene is worth while, the next question is 'How can it be done'?

This problem is simple; all the materials are ready to hand. The teachers are there, so are the schools and the organisation of the Educational department.

All teachers should be given a compulsory course of instruction in elementary hygiene; they should not only be given a good practical knowledge of the subject, but they should be taught how to teach it.

The training schools for teachers already exist; it is easy to fit elementary hygiene into the curriculum.

Hygiene 'readers' are required for the school children or lessons in hygiene can be introduced into the existing readers without the slightest dislocation of the present courses of instruction.

It seems incredible that a subject that has so essential a bearing on the lives and welfare of the people should be almost ignored in our schools and colleges.

It would almost appear as if our educational system is more influenced by the slavery of custom and tradition than is the villager whose conservatism we so complacently look down upon. I consider that the time has come when a strong representative committee should be appointed to consider the best means of ensuring that every scholar and student in the province should receive instruction in hygiene suited to his capacity.



Hygiene is a progressive subject, and one function of the Bureau of Public Health, which every province will soon possess, should be to act as one of the intelligence branches of the Educational department.

The greatest defect of the Educational scheme which has been outlined above is that it will not produce immediate results. This shortcoming is freely admitted, but there is no scheme in the field which is likely to yield both rapid and satisfactory results.

The other suggestions contribute a kind of guerilla warfare which will produce local impressions of a temporary nature. What is needed is an organised campaign which can only be carried on by a properly organised and properly trained army. At first the results will be slow in coming; the habits of generations are not easily thrown aside; but when once the truth of the new ideas begins to prevail, the rate of progress may surprise even the most hopeful.

In connection with the hook worm disease the proper line of action is clear and definite *up to a certain point*; it will take some years to reach that point, and by the time it has been reached the path of further progress will have become clearly defined.

In the meantime there should be no delay in taking the first step. The work of laying foundations is slow and dull, but it is a necessary antecedent to the construction of a sound building.

#### *Summary.*

Hook worm disease is extremely prevalent in the United Provinces.

It causes many deaths every year. It causes some degree of mental and physical deterioration in an enormous number of individuals.

In the aggregate there is a very great economic loss to the province. The disease is easy to recognise and easy to cure.

If the doctors of the province are provided with the means of diagnosing the disease and of treating it, much good will be done.

Instructions by leaflets, lectures, etc., will do some good and should be carried out.

The extension of the use of latrines should be encouraged. Experimental work should be done in the prisons and elsewhere to find out to what extent prevention and cure of the disease are practicable. Similar experimental work should be done in selected infected areas.



Concerted action all over the province cannot be carried out with any prospect of success until the people as a whole have been educated to understand the rudiments of hygiene.

The only means by which the people can be taught the language and ideas of hygiene is by making the subject compulsory in the schools and colleges.

Great credit is due to Dr. Rai and Dr. Banerji for the careful and intelligent manner in which they have carried out the investigation. They have not confined themselves to the lines of inquiry that were prescribed, but in many cases have gone into points which appeared to them to be of importance. In this way they have added greatly to the value of the results obtained. Their work has involved much labour under difficulties and much hardship, but they have the satisfaction of knowing that they have carried out an important public duty with success and credit.

TABLE I.

*Statement of infection in certain districts.*

Name of district.					Number examined.	Number infected.	Percentage of infected.
Saharanpur	..	..	..	..	118	105	88.98
Meerut	..	..	..	..	239	178	74.47
Aligarh	..	..	..	..	230	159	69.13
Agra	..	..	..	..	185	63	34.05
Jhansi	..	..	..	..	95	7	7.36
Banda	..	..	..	..	151	34	22.51
Cawnpore	..	..	..	..	248	75	30.2
Fatehgarh	..	..	..	..	307	179	58.3
Budaun	..	..	..	..	405	327	80.7
Moradabad	..	..	..	..	351	272	77.49
Etawah	..	..	..	..	249	93	37.34
Almora	..	..	..	..	22	19	88.36
Gorakhpur	..	..	..	..	364	331	90.93
Azamgarh	..	..	..	..	166	153	92.77
Jaunpur	..	..	..	..	148	132	89.18
Ghazipur	..	..	..	..	157	144	91.71
Ballia	..	..	..	..	61	52	85.24
Benares	..	..	..	..	314	234	74.52
Mirzapur	..	..	..	..	171	137	80.11
Fyzabad	..	..	..	..	235	206	87.65
Bare Banki	..	..	..	..	129	113	86.59
Rae Bareli	..	..	..	..	194	163	84.02
Sitapur	..	..	..	..	748	649	86.76
Shahjahanpur	..	..	..	..	429	355	82.75
Gonda	..	..	..	..	405	345	85.18
Total					6,121	4,525	73.94

TABLE II.

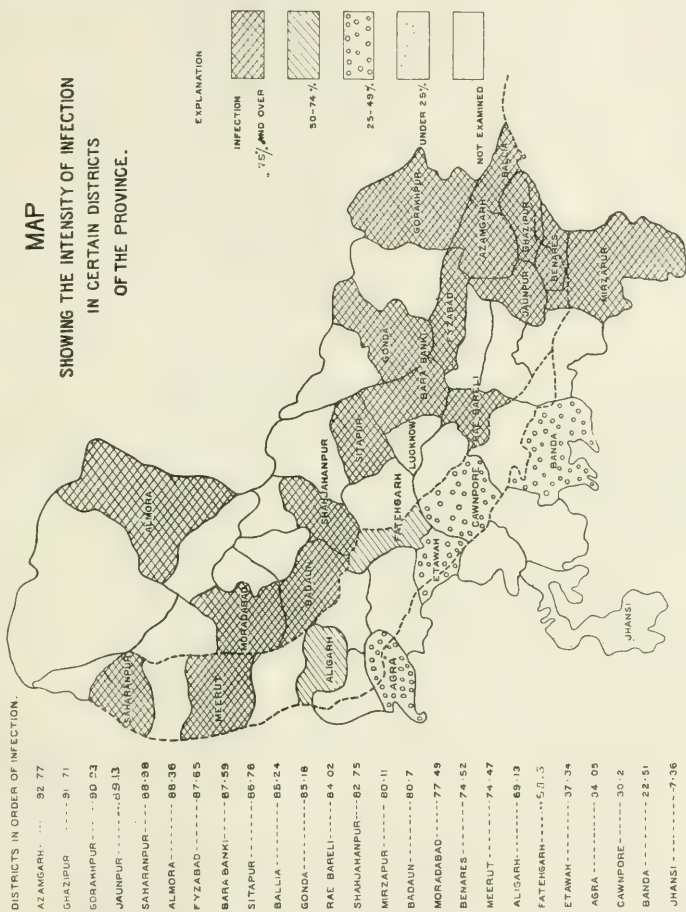
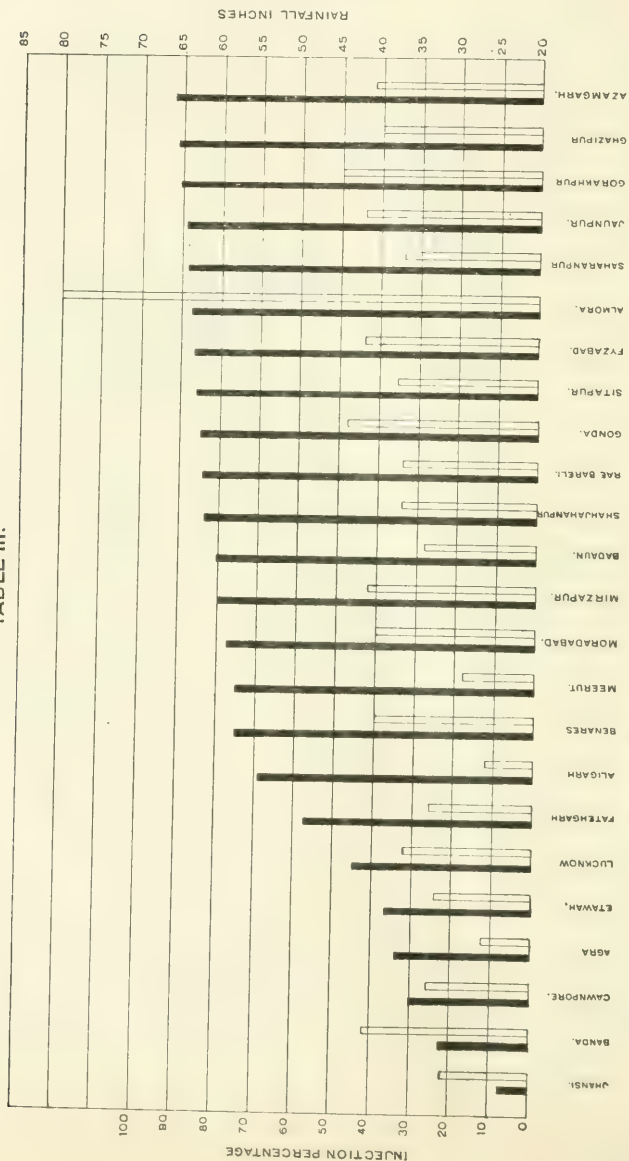


TABLE III.



Graphic representation of percentage infection compared with rainfall.

Solid columns show infection rate.

Hollow columns show rainfall.

TABLE IV.

*Statement showing the effects of stay in jails on hook worm infection.*

UNDER 14 DAYS.			OVER 14 DAYS AND UNDER 1 MONTH.			OVER 1 MONTH AND UNDER 3 MONTHS.			ABOVE 3 MONTHS.		
Examined.	Infect- ed.	Per cent.	Examined.	Infect- ed.	Per cent.	Examined.	Infect- ed.	Per cent.	Examined.	Infect- ed.	Per cent.
953	755	79.22	916	711	77.62	1,765	1,276	72.29	2,487	1,783	71.6

TABLE V.

*Table showing the relation between the degree of infection and the state of health of the prisoners. (Dr. Banerji.)*

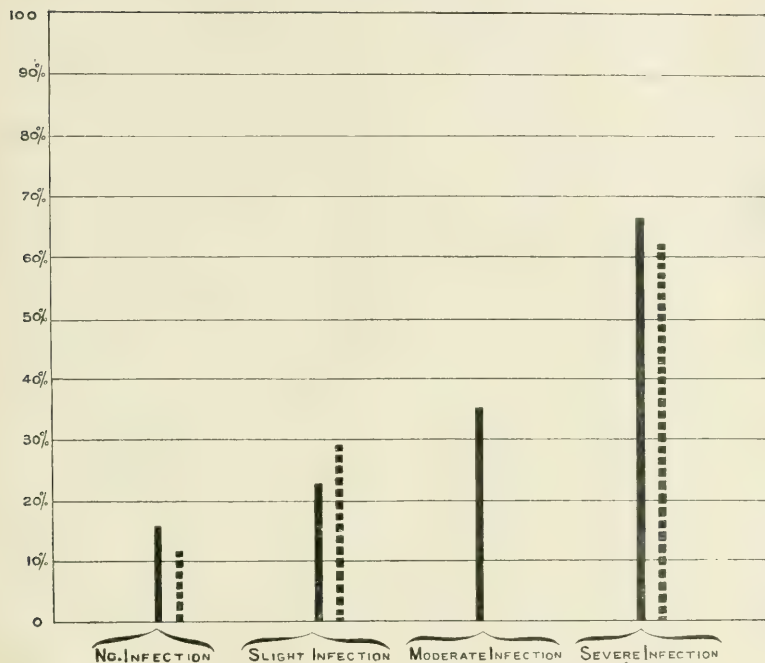
Degree of infection.	Total number examin- ed.	CONDITION OF HEALTH AS ESTIMATED BY EXAMINATION OF THE PRISONER.								APPARENT ANÆMIA.	
		' Good.'		' Indifferent.'		' Bad.'		NO APPARENT ANÆMIA.			
		Total.	Percent- age.	Total.	Percent- age.	Total.	Percent- age.	Total.	Percent- age.	Total.	Percent- age.
No infection . .	1,089	911	83.65	174	15.9	4	.36	958	87.9	131	12.03
Slight infection . .	1,313	1,010	76.9	290	22.08	13	.09	1,090	83.01	223	16.9
Moderate infection .	174	113	64.9	59	33.8	2	1.14	148	85.05	26	14.9
Severe infection . .	24	8	33.3	16	66.6	..	..	15	62.5	9	37.5
Total for all degrees of infection . .	1,511	1,131	74.84	365	24.15	15	.099	1,253	82.92	258	17.07

TABLE VI.

*Table showing relation between the degree of infection and the state of health of the prisoners (Dr. Rai.)*

Degree of infection.	Total number examined.	CONDITION OF HEALTH AS SHOWN ON THE PRISONER'S HISTORY TICKET.						NO APPARENT ANÆMIA.		APPARENT ANÆMIA.	
		' Good.'		' Indifferent.'		' Bad.'					
		Total.	Percent- age.	Total.	Percent- age.	Total.	Percent- age.	Total.	Percent- age.	Total.	Percent- age.
No infection . . .	507	453	89·34	47	9·27	7	1·38	492	97·04	15	2·95
Slight infection . .	2,947	2,072	70·30	826	28·02	49	1·66	2,819	95·65	128	4·34
Severe infection . .	67	25	37·31	39	58·20	3	4·47	41	61·19	26	38·05
Total for all degrees of infection . .	3,014	2,097	69·57	865	28·69	52	1·72	2,860	94·89	154	5·10

TABLE VII.



Graphic representation of the effect on health caused by infection.

The height of the column shows the relative proportion of those in 'bad' and 'indifferent' health in each class.

{ Dr. Banerji's findings in continuous line. }  
 { Dr. Rai's findings in dotted line. }

TABLE VIII.  
*Comparative statement of infection in rural and urban population.*

District.	RURAL.			URBAN.		
	Total examined.	Number found infected.	Percent- age.	Total examined.	Number found infected.	Percent- age.
Saharanpur .	102	94	92.1	16	11	68.7
Meerut . .	223	172	77.1	16	6	37.5
Aligarh . .	211	154	72.9	19	5	26.3
Almora . .	22	19	88.3	..	..	..
Agra . .	125	51	40.8	60	12	20
Jhansi . .	71	6	8.4	24	1	4.16
Banda . .	146	33	22.6	5	1	20
Cawnpore .	78	21	26.9	170	54	31.7
Fatehgarh .	277	170	61.3	30	9	30
Budaun . .	397	324	81.6	8	3	37.5
Moradabad .	318	247	77.6	33	25	75.7
Etawah . .	156	62	39.7	93	31	33.3
Gorakhpur .	302	299	99.006	62	32	51.61
Azamgarh .	150	146	97.33	16	7	43.75
Jaunpur . .	127	123	96.85	21	9	42.85
Ghazipur . .	137	133	97.08	20	11	55.00
Ballia . .	53	48	90.56	8	4	50.00
Benares . .	229	206	89.94	85	28	32.94
Mirzapur . .	138	125	90.57	33	12	36.33
Fyzabad . .	205	193	94.14	30	13	43.33
Bara Banki .	122	109	89.34	7	4	57.14
Rae Bareilly .	179	155	86.59	15	8	53.33
Sitapur . .	722	636	88.08	26	13	50.00
Shahjahanpur .	391	337	86.18	38	18	47.31
Gonda . .	394	339	86.04	11	6	54.54
Total .	5,275	4,202	79.46	846	323	38.17



TABLE IX.

*Table showing the influence of occupation on hook worm infection.*  
*(Dr. Rai and Dr. Banerji.)*

Occupation.	Total examined.	Total infected.	Per- centage.
Field workers . . . . .	2,233	1,911	85.57
Beggars . . . . .	69	49	71.01
Wandering tribes . . . . .	89	59	66.29
Artisans . . . . .	386	238	61.65
Sweepers . . . . .	70	45	64.28
Persons in close contact with water (boat-men, etc.)	83	49	59.03

## EDITORIAL NOTES.

THE Editors direct the attention of Indian research workers to the conditions attached to the Darbhanga Medical Research Scholarship. Further particulars can be obtained on application to the Secretary, School of Tropical Medicine, Calcutta.

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### CALCUTTA SCHOOL OF TROPICAL MEDICINE.

#### THE DARBHANGA MEDICAL RESEARCH SCHOLARSHIP.

THE Darbhanga Medical Research Scholarship at the Calcutta School of Tropical Medicine has been endowed by His Highness the Maharajah Bahadur of Darbhanga. The scholarship is of the value of Rs. 200 p.m. and, will ordinarily be held for three years, under the following conditions :—

- (1) The holder shall be a pure native of India, holding a medical qualification registrable under any Indian Provincial Medical Council. Assistant and Sub-Assistant Surgeons in government service shall also be eligible.
- (2) The scholarship shall be tenable at the Calcutta School of Tropical Medicine, where the research scholar shall ordinarily work.
- (3) Candidates will be required to produce evidence of previous laboratory and scientific training and of proficiency in or aptitude for research work.
- (4) The holder of the scholarship shall work for not less than five hours daily during week days solely at research work during fixed hours in the laboratory.
- (5) The holder shall be appointed by the Governing Body of the Endowment Fund of the Calcutta School of Tropical Medicine. He will be required to submit an annual written report on the work done, or as called upon to do so,

- (6) The scholarship shall ordinarily be tenable for three years, but may be discontinued by the Governing Body at any time if they consider that the holder is not doing satisfactory work. It shall also be open to the Governing Body to extend the term of tenure to a longer period than three years.
- (7) In the event of an Assistant Surgeon or Sub-Assistant Surgeon being appointed it is hoped that the Provincial Government concerned will grant the necessary leave on deputation and will also pay the candidate's grade pay in addition to his receiving the scholarship of Rs. 200 p.m.
- (8) The subject for investigation in the first instance shall be the methods of prevention and cure of filariasis, elephantiasis and allied conditions.
- (9) Applications should reach the Secretary, Calcutta School of Tropical Medicine, Calcutta, before 15th November, 1920, and be supported by true copies of original testimonials, etc.



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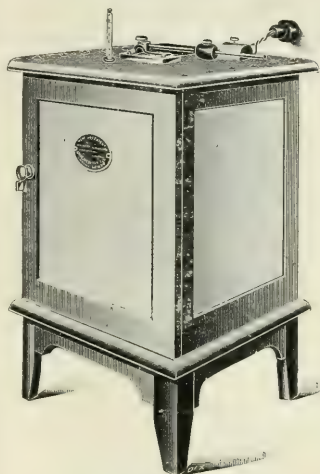
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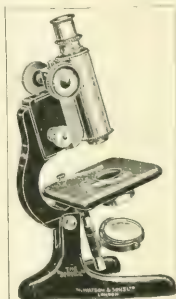
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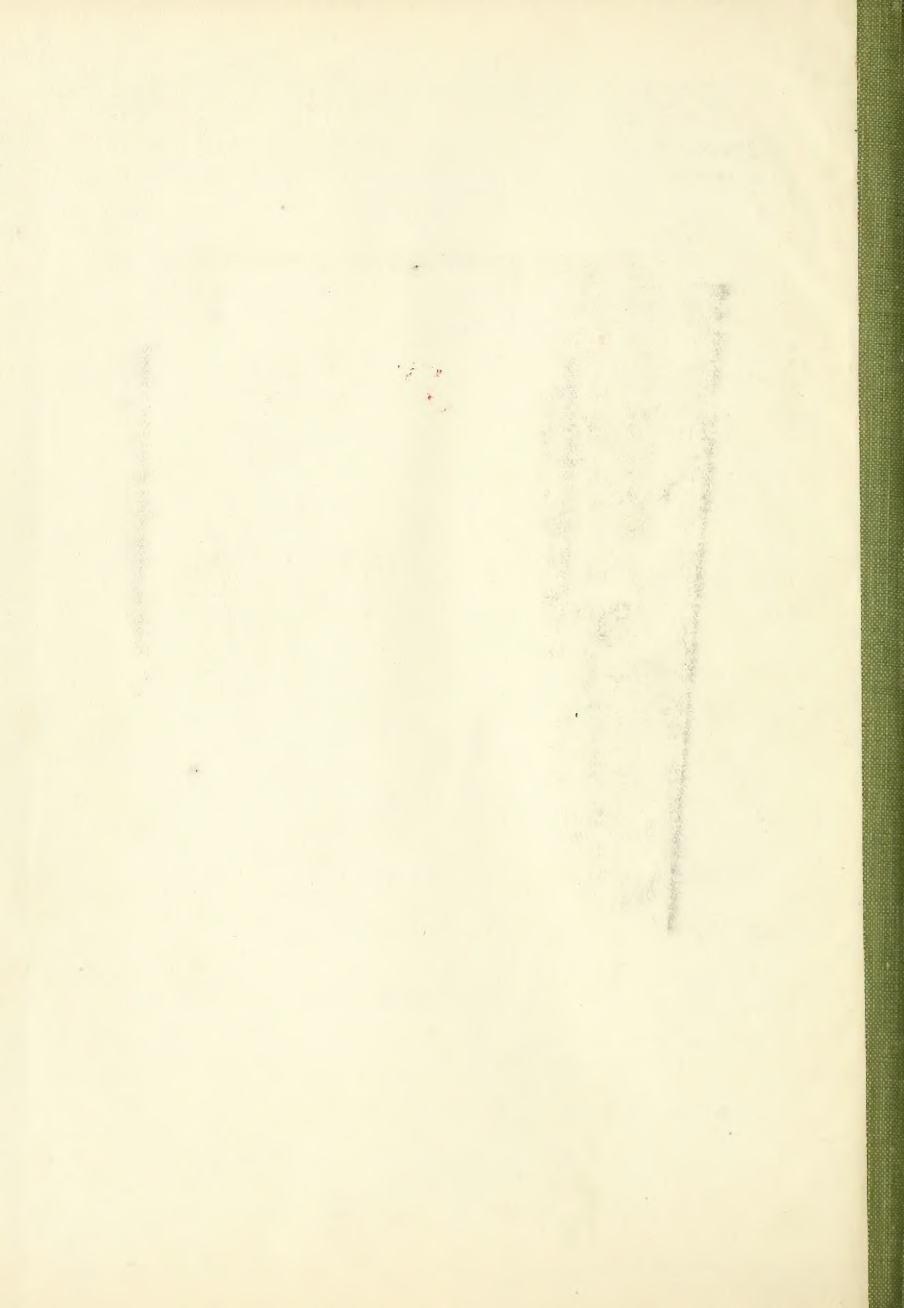
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